PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PHM 70251/WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB98/02259	28/07/1998	01/08/1997
International Patent Classification (IPC) or national classification and IPC C12N15/00		
Applicant ZENECA LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08/02/1999	03. 11. 99
Name and mailing address of the international preliminary examining authority European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx. 523656 epmu d Fax +49 89 2399 - 4465	Authorized officer Fotaki, M Telephone No. +49 89 2399 8709

Form PCT IPEA 409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB98/02259

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-13 as originally filed

Claims, No.:

1-21 as originally filed

Drawings, sheets:

1/19-19/19 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

☐ copy of the earlier application whose priority has been claimed.

☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 14,15, 16.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☒ the claims, or said claims Nos. 14 are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 15, 16.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3, 12, 13, 17-20
	No:	Claims	1, 2, 4-11, 21
Inventive step (IS)	Yes:	Claims	none
	No:	Claims	1-13, 17-21
Industrial applicability (IA)	Yes:	Claims	1-13, 17, 18, 20, 21
	No:	Claims	19 (reserved opinion)

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

II. PRIORITY

1) This first preliminary written opinion has been established considering the priority date 01.08.97 as a valid date. The Applicant is reminded that documents:
WO 97 37223 A (UNIV NORTH CAROLINA) 9 October 1997
OHARA O. ET AL. in EMBL DATABASE, 5 December 1997
cited in the international search report may become relevant after consideration of the priority document which is unavailable at present.

III. NON-ESTABLISHMENT OF OPINION

2) The subject-matter of **Claim 14** is not supported by the description and thus, it is not amenable to examination of novelty, inventive step or industrial applicability. Said claim relates to a method for identifying a compound capable of modulating the activity of a ZGGBP1 protein. However, a function of said protein has not been demonstrated. Sequence analysis of the encoding gene has revealed several structural domains which correspond to several potential functions (p. 2). Furthermore, sequence homology of the encoding gene with at least two different genes, nedd-4 and Pub3, indicates even more potential functions (p.3). The application as filed, does not provide any indication as to which of all the potential activities of ZGGBP1 the method of **Claim 14** is directed to. Since the method of said claim is not supported by the description, an opinion cannot be established .

3) No opinion is established for the subject-matter of **Claims 15 and 16** because said claims relate to inventions in respect of which no international search report has been established (Rule 66.1(e) PCT).

V. REASONED STATEMENT UNDER ARTICLE 35(2)

4) The present application relates to the isolation of a cDNA clone comprising the sequence presented in SEQ ID NO1, called gene ZGGBP1. Said sequence resides, along with many other expressed sequences, in the 18q21 chromosomal region which was shown previously to be associated with the neurological disorder bipolar affective disorder. The ZGGBP1 gene sequence appears to have 85% identity at the amino acid level with the human gene encoding nedd-4 which

was shown previously to be a negative regulator of a sodium channel which is deleted in Liddle's syndrome (a hereditary form of hypertension). The ZGGBP1 gene sequence shares also 100% identity, over a 3kb region, with the previously identified gene, Pub3 which may be involved in regulation of cell cycle of eukaryotic cells. The Applicant has not demonstrated that said ZGGBP1 gene has any function.

5) The subject-matter of **Claims 1, 2, 4-11, 21** is not novel as required by Article 33(2) PCT.

Claim 1 relates to a polynucleotide comprising a nucleic acid sequence encoding the polypeptide of SEQ ID NO 2, and homologues and fragments.

Figure 2 of the present application discloses the amino acid sequence of the known human nedd-4 gene which is 85% homologous to polypeptide of SEQ ID NO 2 (p.11). Figure 5 of the present application discloses the nucleotide sequence of the known gene Pub-3 which comprises fragments up to 3 kb long which are identical to the polynucleotide encoding SEQ ID NO 2.

Thus, the subject-matter of said claim has been disclosed in the prior art.

Similar arguments apply for the subject-matter of **Claims 2, 4-11, 21**.

6) The subject-matter of **Claims 3, 12, 13, 17-20** is not inventive as required by Article 33(3) PCT.

Said claims refer to subject-matter as defined in **Claims 1-7, 10 or 11** and comprise additional technical features which may render said subject-matter novel. However, said technical features represent standard options available to the skilled person aware of the state of the art in the field of gene technology. Thus, said technical features do not impart any inventiveness to said subject-matter. Consequently, even if it is assumed that the subject-matter of **Claims 3, 12, 13, 17-20** is novel, said claims do not comprise an inventive step.

7) **Claim 19** is directed to the use of a polynucleotide in gene therapy. Said claim is

thus, considered to be directed to method of treatment of the human or animal body. For the assessment of claims as far as they are directed to a method of treatment of the human or animal body or to a diagnostic method practised on the human or animal body, no unified criteria exist in the PCT, on the question whether they are industrially applicable. The patentability can be dependent upon the formulation of the claims.

VI. CERTAIN DOCUMENTS CITED

8) The following documents are cited under Rule 70.10 PCT
WO 97 37223 A, 9.10.97, filed 03.04.97, with priority date of 03.04.96

VIII. CERTAIN OBSERVATIONS ON THE INTERNATIONAL APPLICATION

9) The Applicant is reminded that the claims must be comprehensible from the technical point of view and clearly define the object of the invention, that is to say indicate all the essential features thereof (Rule 6 PCT). The subject-matter of **Claims 3, 7, 17 and 21** does not fulfil this condition, as the claimed nucleic acid is only defined by the name of the encoded protein "ZGGBP1" without disclosing any technical feature which unambiguously characterizes the claimed subject-matter. A gene being a chemical product should be clearly defined by its formula i.e. its nucleotide sequence.

10) **Claim 1** is drafted towards an isolated nucleic acid with specified sequence content. However, the function of said nucleic acid is not clearly stated in the claim.

The Applicant speculates in the description that said nucleic acid encodes a protein which may be associated with the bipolar affective disorder although no evidence for such association is presented. Furthermore, even if such an association of the claimed protein with the bipolar affective disorder is established, a function of the protein is not defined nor could be speculated, especially since sequence analysis of the protein appears to indicate several potential functions. Since there is no conclusive demonstration of any function of the polypeptide of SEQ ID NO 2, as such, any function can, at best, be accepted as speculative. At

this point, it is not apparent what problem said polypeptide or fragments thereof are intended to solve or whether said sequences represent a solution comprising an inventive step. Insufficient disclosure of essential technical features, such as the function of a given nucleotide or amino acid sequence, does not allow for the acknowledgement of an inventive step involved in solving a technical problem. The lack of sufficient disclosure of the invention is contrary to Article 5 PCT and makes it difficult if not impossible to examine the novelty and inventive step of the claimed invention.

The same arguments apply to the subject-matter of **Claims 2-7 and 10-12**.

11) The subject-matter of **Claim 11** is not clear as required by Rule 6 PCT. Said claim relates to a polypeptide comprising the amino acid sequence of SEQ ID NO 4. As said sequence is being disclosed only in the sequence listing without any mention of it in the description, it is not entirely clear what relation it bears with the invention as disclosed in the present application.

INTERNATIONAL SEARCH REPORT

Internat. . Application No
PCT/GB 98/02259

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/00 C07K14/435 C12N9/10 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X,P	WO 97 37223 A (UNIV NORTH CAROLINA) 9 October 1997	6,10, 12-14, 18-21
A	see abstract see page 9, line 1 - page 10, line 23 see figure 23 see claim 48 see Nos.125 and 126 of Sequence Listing ---	1,2,4
X,P	OHARA O. ET AL.: "Prediction of the sequences of unidentified human genes. VIII. The complete senquences of 77 new cDNA clones from brain which can code for large proteins in vitro" EMBL DATABASE,5 December 1997, XP002087609 HEIDELBERG, DE AC: AB007899 --- -/--	1,2,4, 8-10,18, 21

[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex

° Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 December 1998	12/01/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P B 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx 31 651 epo nl, Fax: (+31-70) 340-3016	Panzica, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internat ...l Application No
PCT/GB 98/02259

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	STINE O.C. ET AL.: "Evidence for linkage of bipolar disorder to chromosome 18 with a parent-of-origin effect" AMERICAN JOURNAL OF HUMAN GENETICS, vol. 57, no. 6, 1995, pages 1384-1394, XP002087610 US cited in the application see the whole document ---	
A	MORS O. ET AL.: "Cytogenetic abnormalities on chromosome 18 associated with bipolar affective disorder or schizophrenia" BRITISH JOURNAL OF PSYCHIATRY, vol. 170, March 1997, pages 278-280, XP002087611 GB -----	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 98/02259

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons.

1 [ ] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. [X] Claims Nos.: CLAIMS 15, 16
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

SEE FURTHER INFORMATION SHEET PCT/ISA/210

3 [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1 [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2 [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3 [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4 [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/ GB 98/02259

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

CLAIMS NOS.: 15, 16

A search for the claims 15 and 16, respectively relating to a compound modulating the activity of the protein of claims 1 and 2 and to a substance modulating the compound identified by the method of claim 14, could not be performed, since the subject-matter is not sufficiently disclosed.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat l Application No
PCT/GB 98/02259

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9737223 A	09-10-1997	AU 2659797 A	22-10-1997

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* PHM 70251/WO

Box No. I TITLE OF INVENTION

NOVEL COMPOUNDS

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

ZENECA Limited
15 Stanhope Gate
London
GB-W1Y 6LN
GB

☐ This person is also inventor.

Telephone No.
(01625) 516173

Facsimile No.
(01625) 583358

Teleprinter No.
669095/669388

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

FLANNERY, Angela Veronica
Alderley Park
Macclesfield
Cheshire
GB-SK10 4TG
GB

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

PHILLIPS, Neil Godfrey Alasdair
Intellectual Property Department
ZENECA Pharmaceuticals
Mereside, Alderley Park
Macclesfield, Cheshire, GB-SK10 4TG, GB

Telephone No.
(01625) 514304

Facsimile No.
(01625) 583358

Teleprinter No.
669095/669388

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Sheet No. 2

Continuation of Box No. III FUR[redacted]PLICANT(S) AND/OR (FURTHER) INVENT[redacted]

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

FINNEGAN, Maria Christina Martina
Alderley Park
Macclesfield
Cheshire
GB-SK10 4TG
GB

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: IE

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [X] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

[ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- ☐ **AP** **ARIPO Patent:** **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SZ** Swaziland, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☐ **EA** **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakhstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP** **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☐ **OA** **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- ☐ **AL** Albania .....
- ☐ **AM** Armenia .....
- ☐ **AT** Austria .....
- ☐ **AU** Australia .....
- ☐ **AZ** Azerbaijan
- ☐ **BA** Bosnia and Herzegovina .....
- ☐ **BB** Barbados
- ☐ **BG** Bulgaria .....
- ☐ **BR** Brazil .....
- ☐ **BY** Belarus .....
- ☐ **CA** Canada
- ☐ **CH and LI** Switzerland and Liechtenstein
- ☐ **CN** China .....
- ☐ **CU** Cuba .....
- ☐ **CZ** Czech Republic .....
- ☐ **DE** Germany .....
- ☐ **DK** Denmark .....
- ☐ **EE** Estonia .....
- ☐ **ES** Spain .....
- ☐ **FI** Finland .....
- ☐ **GB** United Kingdom
- ☐ **GE** Georgia .....
- ☐ **GH** Ghana .....
- ☐ **GM** Gambia
- ☐ **GW** Guinea-Bissau .....
- ☐ **HR** Croatia
- ☐ **HU** Hungary .....
- ☐ **ID** Indonesia
- ☐ **IL** Israel .....
- ☐ **IS** Iceland
- ☒ **JP** Japan .....
- ☐ **KE** Kenya .....
- ☐ **KG** Kyrgyzstan .....
- ☐ **KP** Democratic People's Republic of Korea .....
- ☐ **KR** Republic of Korea .....
- ☐ **KZ** Kazakhstan .....
- ☐ **LC** Saint Lucia
- ☐ **LK** Sri Lanka
- ☐ **LR** Liberia
- ☐ **LS** Lesotho .....
- ☐ **LT** Lithuania
- ☐ **LU** Luxembourg
- ☐ **LV** Latvia
- ☐ **MD** Republic of Moldova .....
- ☐ **MG** Madagascar .....
- ☐ **MK** The former Yugoslav Republic of Macedonia .....
- ☐ **MN** Mongolia
- ☐ **MW** Malawi .....
- ☐ **MX** Mexico .....
- ☐ **NO** Norway
- ☐ **NZ** New Zealand .....
- ☐ **PL** Poland .....
- ☐ **PT** Portugal .....
- ☐ **RO** Romania
- ☐ **RU** Russian Federation .....
- ☐ **SD** Sudan
- ☐ **SE** Sweden
- ☐ **SG** Singapore
- ☐ **SI** Slovenia .....
- ☐ **SK** Slovakia .....
- ☐ **SL** Sierra Leone
- ☐ **TJ** Tajikistan .....
- ☐ **TM** Turkmenistan .....
- ☐ **TR** Turkey .....
- ☐ **TT** Trinidad and Tobago .....
- ☐ **UA** Ukraine .....
- ☐ **UG** Uganda .....
- ☒ **US** United States of America .....
- ☐ **UZ** Uzbekistan .....
- ☐ **VN** Viet Nam .....
- ☐ **YU** Yugoslavia .....
- ☐ **ZW** Zimbabwe .....

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- ☐ .....
- ☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM ☐ Further priority claims are [redacted]ated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 01 August 1997 (01.08.97)	9716162.4	GB		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): item (1)

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*: ISA /

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*:
Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	:	4
description (excluding sequence listing part)	:	13
claims	:	3
abstract	:	1
drawings	:	19
sequence listing part of description	:	16
Total number of sheets	:	56

This international application is **accompanied by** the item(s) marked below:

1. ☒ fee calculation sheet
2. ☒ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☒ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other *(specify)*:

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Neil Phillips

Neil Godfrey Alasdair PHILLIPS
AGENT

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
PHM 70251/WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 98/02259	28/07/1998	01/08/1997
Applicant		
ZENECA LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. ☒ **Certain claims were found unsearchable** (see Box I).

2. ☐ **Unity of invention is lacking** (see Box II).

3. ☒ The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - ☒ filed with the international application.
   - ☐ furnished by the applicant separately from the international application.
     - ☐ but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - ☐ Transcribed by this Authority

4. With regard to the **title,**
   - ☐ the text is approved as submitted by the applicant
   - ☒ the text has been established by this Authority to read as follows:

ZGGBP1, NOVEL PEPTIDES RELATED TO BIPOLAR AFFECTIVE DISORDER TYPE 1, SEQUENCES AND USES THEREOF

5. With regard to the **abstract,**
   - ☒ the text is approved as submitted by the applicant
   - ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

Figure No. _____
- ☐ as suggested by the applicant.
- ☐ because the applicant failed to suggest a figure.
- ☐ because this figure better characterizes the invention.
- ☒ None of the figures

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 98/02259

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: CLAIMS 15, 16
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

SEE FURTHER INFORMATION SHEET PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

CLAIMS NOS.: 15, 16

A search for the claims 15 and 16, respectively relating to a compound modulating the activity of the protein of claims 1 and 2 and to a substance modulating the compound identified by the method of claim 14, could not be performed, since the subject-matter is not sufficiently disclosed.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claims Nos.: 15 16

A search for the claims 15 and 16, respectively relating to a compound modulating the activity of the protein of claims 1 and 2 and to a substance modulating the the compound identified by the method of claim 14, could not be performed, since the subject-matter is not sufficiently disclosed.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/[redacted] 98/02259

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/00 C07K14/435 C12N9/10 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 97 37223 A (UNIV NORTH CAROLINA) 9 October 1997	6,10, 12-14, 18-21
A	see abstract see page 9, line 1 - page 10, line 23 see figure 23 see claim 48 see Nos.125 and 126 of Sequence Listing ---	1,2,4
X,P	OHARA O. ET AL.: "Prediction of the sequences of unidentified human genes. VIII. The complete senquences of 77 new cDNA clones from brain which can code for large proteins in vitro" EMBL DATABASE,5 December 1997, XP002087609 HEIDELBERG, DE AC: AB007899 --- -/--	1,2,4, 8-10,18, 21

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 December 1998	12/01/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Panzica, G

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	STINE O.C. ET AL.: "Evidence for linkage of bipolar disorder to chromosome 18 with a parent-of-origin effect" AMERICAN JOURNAL OF HUMAN GENETICS, vol. 57, no. 6, 1995, pages 1384-1394, XP002087610 US cited in the application see the whole document ---	
A	MORS O. ET AL.: "Cytogenetic abnormalities on chromosome 18 associated with bipolar affective disorder or schizophrenia" BRITISH JOURNAL OF PSYCHIATRY, vol. 170, March 1997, pages 278-280, XP002087611 GB -----	

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 98/02259

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9737223 A	09-10-1997	AU 2659797 A	22-10-1997

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PHM 70251/WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB98/02259	28/07/1998	01/08/1997
International Patent Classification (IPC) or national classification and IPC C12N15/00		
Applicant ZENECA LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08/02/1999	03. 11. 99
Name and mailing address of the international preliminary examining authority European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx. 523656 epmu d Fax +49 89 2399 - 4465	Authorized officer Fotaki, M Telephone No. +49 89 2399 8709

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB98/02259

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-13 as originally filed

Claims, No.:

1-21 as originally filed

Drawings, sheets:

1/19-19/19 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

☐ copy of the earlier application whose priority has been claimed.

☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 14,15, 16.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☒ the claims, or said claims Nos. 14 are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 15, 16.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3, 12, 13, 17-20
	No:	Claims	1, 2, 4-11, 21
Inventive step (IS)	Yes:	Claims	none
	No:	Claims	1-13, 17-21
Industrial applicability (IA)	Yes:	Claims	1-13, 17, 18, 20, 21
	No:	Claims	19 (reserved opinion)

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

II. PRIORITY

1) This first preliminary written opinion has been established considering the priority date 01.08.97 as a valid date. The Applicant is reminded that documents:
WO 97 37223 A (UNIV NORTH CAROLINA) 9 October 1997
OHARA O. ET AL. in EMBL DATABASE, 5 December 1997
cited in the international search report may become relevant after consideration of the priority document which is unavailable at present.

III. NON-ESTABLISHMENT OF OPINION

2) The subject-matter of **Claim 14** is not supported by the description and thus, it is not amenable to examination of novelty, inventive step or industrial applicability. Said claim relates to a method for identifying a compound capable of modulating the activity of a ZGGBP1 protein. However, a function of said protein has not been demonstrated. Sequence analysis of the encoding gene has revealed several structural domains which correspond to several potential functions (p. 2). Furthermore, sequence homology of the encoding gene with at least two different genes, nedd-4 and Pub3, indicates even more potential functions (p.3). The application as filed, does not provide any indication as to which of all the potential activities of ZGGBP1 the method of **Claim 14** is directed to. Since the method of said claim is not supported by the description, an opinion cannot be established .

3) No opinion is established for the subject-matter of **Claims 15 and 16** because said claims relate to inventions in respect of which no international search report has been established (Rule 66.1(e) PCT).

V. REASONED STATEMENT UNDER ARTICLE 35(2)

4) The present application relates to the isolation of a cDNA clone comprising the sequence presented in SEQ ID NO1, called gene ZGGBP1. Said sequence resides, along with many other expressed sequences, in the 18q21 chromosomal region which was shown previously to be associated with the neurological disorder bipolar affective disorder. The ZGGBP1 gene sequence appears to have 85% identity at the amino acid level with the human gene encoding nedd-4 which

was shown previously to be a negative regulator of a sodium channel which is deleted in Liddle's syndrome (a hereditary form of hypertension). The ZGGBP1 gene sequence shares also 100% identity, over a 3kb region, with the previously identified gene, Pub3 which may be involved in regulation of cell cycle of eukaryotic cells. The Applicant has not demonstrated that said ZGGBP1 gene has any function.

5) The subject-matter of **Claims 1, 2, 4-11, 21** is not novel as required by Article 33(2) PCT.

Claim 1 relates to a polynucleotide comprising a nucleic acid sequence encoding the polypeptide of SEQ ID NO 2, and homologues and fragments.

Figure 2 of the present application discloses the amino acid sequence of the known human nedd-4 gene which is 85% homologous to polypeptide of SEQ ID NO 2 (p.11). Figure 5 of the present application discloses the nucleotide sequence of the known gene Pub-3 which comprises fragments up to 3 kb long which are identical to the polynucleotide encoding SEQ ID NO 2.

Thus, the subject-matter of said claim has been disclosed in the prior art.

Similar arguments apply for the subject-matter of **Claims 2, 4-11, 21**.

6) The subject-matter of **Claims 3, 12, 13, 17-20** is not inventive as required by Article 33(3) PCT.

Said claims refer to subject-matter as defined in **Claims 1-7, 10 or 11** and comprise additional technical features which may render said subject-matter novel. However, said technical features represent standard options available to the skilled person aware of the state of the art in the field of gene technology. Thus, said technical features do not impart any inventiveness to said subject-matter. Consequently, even if it is assumed that the subject-matter of **Claims 3, 12, 13, 17-20** is novel, said claims do not comprise an inventive step.

7) **Claim 19** is directed to the use of a polynucleotide in gene therapy. Said claim is

thus, considered to be directed to method of treatment of the human or animal body. For the assessment of claims as far as they are directed to a method of treatment of the human or animal body or to a diagnostic method practised on the human or animal body, no unified criteria exist in the PCT, on the question whether they are industrially applicable. The patentability can be dependent upon the formulation of the claims.

VI. CERTAIN DOCUMENTS CITED

8) The following documents are cited under Rule 70.10 PCT
WO 97 37223 A, 9.10.97, filed 03.04.97, with priority date of 03.04.96

VIII. CERTAIN OBSERVATIONS ON THE INTERNATIONAL APPLICATION

9) The Applicant is reminded that the claims must be comprehensible from the technical point of view and clearly define the object of the invention, that is to say indicate all the essential features thereof (Rule 6 PCT). The subject-matter of **Claims 3, 7, 17 and 21** does not fulfil this condition, as the claimed nucleic acid is only defined by the name of the encoded protein "ZGGBP1" without disclosing any technical feature which unambiguously characterizes the claimed subject-matter. A gene being a chemical product should be clearly defined by its formula i.e. its nucleotide sequence.

10) **Claim 1** is drafted towards an isolated nucleic acid with specified sequence content. However, the function of said nucleic acid is not clearly stated in the claim.

The Applicant speculates in the description that said nucleic acid encodes a protein which may be associated with the bipolar affective disorder although no evidence for such association is presented. Furthermore, even if such an association of the claimed protein with the bipolar affective disorder is established, a function of the protein is not defined nor could be speculated, especially since sequence analysis of the protein appears to indicate several potential functions. Since there is no conclusive demonstration of any function of the polypeptide of SEQ ID NO 2, as such, any function can, at best, be accepted as speculative. At

this point, it is not apparent what problem said polypeptide or fragments thereof are intended to solve or whether said sequences represent a solution comprising an inventive step. Insufficient disclosure of essential technical features, such as the function of a given nucleotide or amino acid sequence, does not allow for the acknowledgement of an inventive step involved in solving a technical problem. The lack of sufficient disclosure of the invention is contrary to Article 5 PCT and makes it difficult if not impossible to examine the novelty and inventive step of the claimed invention.

The same arguments apply to the subject-matter of **Claims 2-7 and 10-12**.

11) The subject-matter of **Claim 11** is not clear as required by Rule 6 PCT. Said claim relates to a polypeptide comprising the amino acid sequence of SEQ ID NO 4. As said sequence is being disclosed only in the sequence listing without any mention of it in the description, it is not entirely clear what relation it bears with the invention as disclosed in the present application.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 01 April 1999 (01.04.99)	
International application No. PCT/GB98/02259	**Applicant's or agent's file reference** PHM 70251/WO
International filing date (day/month/year) 28 July 1998 (28.07.98)	**Priority date** (day/month/year) 01 August 1997 (01.08.97)
Applicant FLANNERY, Angela, Veronica et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

08 February 1999 (08.02.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer C. Carrié
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/00, C07K 14/435, C12N 9/10, C12Q 1/68

A1

(11) International Publication Number: **WO 99/06539**

(43) International Publication Date: 11 February 1999 (11.02.99)

(21) International Application Number: PCT/GB98/02259

(22) International Filing Date: 28 July 1998 (28.07.98)

(30) Priority Data:
9716162.4 1 August 1997 (01.08.97) GB

(71) Applicant *(for all designated States except US)*: ZENECA LIMITED [GB/GB]; 15 Stanhope Gate, London W1Y 6LN (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: FLANNERY, Angela, Veronica [GB/GB]; Alderley Park, Macclesfield, Cheshire SK10 4TG (GB). FINNEGAN, Maria, Christina, Martina [IE/GB]; Alderley Park, Macclesfield, Cheshire SK10 4TG (GB).

(74) Agent: PHILLIPS, Neil, Godfrey, Alasdair; Zeneca Pharmaceuticals, Intellectual Property Dept., Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB).

(81) Designated States: JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: ZGGBP1, NOVEL PEPTIDES RELATED TO BIPOLAR AFFECTIVE DISORDER TYPE 1, SEQUENCES AND USES THEREOF

(57) Abstract

A new human gene (ZGGBP1) is described which is associated with neurological affective disorders such as bipolar affective disorder. A full-length cDNA encoding human ZGGBP1 and a partial cDNA encoding muringe ZGGBP1 are disclosed. Polymorphic variants of the gene and functional domains encoded within the gene are also provided. The invention further relates to methods for identifying compounds which modulate the activity of ZGGBP1 protein, and to diagnostic assays for the detection of ZGGBP1 in biological samples.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
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DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

ZGGBP1, NOVEL PEPTIDES RELATED TO BIPOLAR AFFECTIVE DISORDER TYPE 1, SEQUENCES AND USES THEREOF

This invention relates to a novel human gene (ZGGBP1) associated with affective neurological disorders such as bipolar affective disorder. The invention also relates to homologues of the ZGGBP1 gene in species such as rat and mouse useful in providing animal models of affective disorders. The invention further relates to both the cDNA and the structural gene and to fragments encoding functional domains within the gene. The invention also relates to means for producing the protein encoded by the gene and to means for regulating its production and activity in vivo.

Affective disorders comprise a broad and heterogeneous category of psychiatric illness with a prevalence of up to 20% in the population. The most severe of these disorders is bipolar type I which affects approximately 1% of the population and this rate is fairly consistent across countries. The disease affects young adults, with a mean age of onset of 22 years. Treatment depends upon the phase of the disease and pharmacological agents include lithium carbonate, carbamazepine or valproic acid, tricyclic antidepressants. Monoamine oxidase inhibitors and selective serotonin re-uptake inhibitors are now also being used. The success rate of individual drugs is variable and some patients are treated with a combination of agents, although most have some unwanted side-effects. At present the precise diagnosis of individual affective disorders is difficult and new, gene based, diagnostic methods are desirable.

Family, twin and adoption studies have suggested the importance of genetic predisposition to bipolar affective disorder. On this basis, several groups have undertaken genetic linkage analysis in families with a high incidence of the disorder to find a causal gene. Many of the studies show conflicting data suggesting that a single gene is unlikely to be the cause. Rather, multiple interacting genetic traits may be involved. A recent study (Stine et al. 1995) identified two regions on chromosome 18 showing linkage to the disease.

The present invention is based on our discovery of a novel gene which maps to 18q21 and which unexpectedly shows appreciable sequence homology to the ned-4 gene on chromosome 15. Ned-4 is the human homologue of the mouse nedd-4 gene which is known to be differentially expressed during neural development and to be involved in signal transduction. Human ned-4 has been shown (Schild et al. 1996, Straub

et al. 1996) to be a negative regulator of a sodium channel which is deleted in Liddle's syndrome (a hereditary form of hypertension).

Nedd-4 was originally isolated as a partial cDNA clone from a mouse brain library (Kumar et al. 1992) as one of a set of genes which were differentially expressed during development (Neural precursor cells expressed developmentally down-regulated). The derived amino acid sequence contains three copies of the WW domain (Andre & Springael 1994, Bork & Sudol, 1994; Hofmann & Boucher, 1995), a Ca lipid binding (CaLB/C2) domain (Brose et al. 1995) and a Hect (homologous to the E6-AP carbodyl terminus) domain which has homology to a ubiquitin ligase (E3) enzyme (Huibregtse et al. 1995). The human homologue of nedd-4 (Ned-4) was isolated as an randomly cloned EST (KIAA0093) from immature myeloblast mRNA (Nomura et al. 1994) and shown by sequence comparison to have 86% identity at the amino acid level to the mouse sequence. The human sequence, however, has a fourth copy of the WW domain.

The WW domain is a 40 amino acid sequence found in several unrelated proteins. The two highly conserved tryptophans give it its name. The function of the domain is thought to be involved in protein-protein interactions. Despite their functional diversity, the proteins listed all appear to be involved in cell signalling or regulation. It has been shown that the WW domains of Nedd-4 interact with the proline-rich PY motifs in the epithelial sodium channel in the kidney (Schild et al. 1996). Mutational deletion of the PY motifs in the epithelium sodium channel in Liddle's syndrome, an inherited disease causing systemic hypertension characterised by hyperactivity of the sodium channel, has been shown to abrogate binding of Nedd-4 (Straub et al. 1996). It is therefore likely that Nedd-4 has a negative regulatory role when bound to the channel.

The Hect domain is an E3 ubiquitin-protein ligase domain and enzymes with this domain catalyse polyubiquitination, which is involved in several cellular processes including proteolytic degradation.

The CaLB/C2 domain is thought to be involved in calcium-dependent phospholipid binding, although some proteins containing this domain do not bind calcium and other putative functions for the C2 domain such as binding to inositol -1,3,4,5-tetraphosphate have been suggested. Examples of proteins containing this domain are Protein Kinase C (PKC) isoenzymes and synaptogamins.

PCT patent application WO97/12962 discloses a protein (Pub3) with homology to Pub1, a Schizosaccaromyces Pombe protein which has an apparent function in the ubiquitination of, among other cellular proteins, the mitotic activating tyrosine phosphatase cdc25 and the tumour suppresser protein p53. As such this protein may be involved in regulating the progression of proliferation in eukaryotic cells by effectively controlling the activity of the cdk complexes by modulating the availability of cdc25 and/or p53.

A comparison of Pub3 with ZGGBP1 revealed that the sequences represent two distinct genes which code for two separate, structurally unrelated proteins. The two genes share sequence homology within a certain defined region, the sequences are identical within the region 516-3568 of ZGGBP1, but they do not show any homology within the regions 5' and 3'of this sequence. In addition the derived amino acid sequence for ZGGBP1 is completely different to that derived for Pub 3 as both have been initiated from a different start methionine. A comparison of the nucleotide sequences for ZGGBP1 and Pub 3 is outlined in Figure 5.

Therefore in a first aspect of the present invention we provide the ZGGBP1 gene having the full length cDNA as set out in SEQ ID NO: 1. We further provide fragments of the ZGGBP1 gene comprising ZGGBP1 sequence outside the region defined by base pairs 516-3568 of the ZGGBP1 gene. By fragments we mean contiguous regions of the gene including complementary DNA and RNA sequences, starting with short sequences useful as probes or primers of say about 8-50 bases, such as 10-30 bases or 15-35 bases, to longer sequences of up to 50, 100, 200, 500 or 1000 bases. Indeed any convenient fragment of the gene of say up to 2kb, 3kb, 4kb or more than 4kb may be a useful gene fragment for further research, therapeutic or diagnostic purposes. Further convenient fragments include those whose terminii are defined by restriction sites within the gene of one or more kinds, such as any combination of Rsa1, Alu1 and Hinf1.

In a further aspect of the invention we provide homologues of the ZGGBP1 gene in species such as rat and mouse useful in providing animal models of affective disorders. By homologue, we mean a corresponding ZGGBP1 gene in another species, which displays greater than 85% sequence homology, conveniently greater than 90%, for example 95%, to the human ZGGBP1 sequence. The full sequences of the individual homologues may be determined using conventional techniques such as hybridisation, PCR

and sequencing techniques, starting with any convenient part of the sequence set out in SEQ ID NO: 1. The partial sequence of the mouse gene is set out in SEQ ID NO: 3 and this gene and the protein encoded by this gene represent further independent aspects of the invention.

In a further aspect of the invention we provide polynucleotide sequences capable of specifically hybridising to the ZGGBP1 gene. By specifically hybridising we mean that the polynucleotide hybridises under stringent conditions to the sequence on chromosome 18q21 as set out in SEQ ID No: 1, or to the corresponding non-coding sequence, to the exclusion of other genomic loci. It is contemplated that a species such as a peptide nucleic acid may be an acceptable equivalent to a polynucleotide, at least for purposes that do not require translation into protein.

In a further aspect of the invention we provide a recombinant ZGGBP1 protein obtained by expression of all or a part of the cDNA as set out in SEQ ID NO: 1. The recombinant protein may comprise all or a convenient part of the peptide sequence set out in SEQ ID NO: 2. The production of a protein according to the invention may be achieved using standard recombinant DNA techniques involving the expression of the protein by a host cell as described for example by Sambrook et al. 1989. The isolated nucleic acids described herein may for example be introduced into any convenient expression vector for example the T7 Studier system for expression in E.coli (US-A-4952496), Pichia pastoris for expression in yeast, the Baculovirus system for expression in insect cells and the GS system for expression in mammalian cells by operatively linking the DNA to any necessary expression control elements therein and transforming any suitable prokaryotic or eukaryotic host cell with the vector using well known procedures.

Therefore in a further aspect of the invention we provide a recombinant plasmid comprising all or a part of the ZGGBP1 cDNA of the invention.

The invention further extends to cells containing said recombinant plasmids and to a process for producing a ZGGBP1 protein of the invention which comprises culturing said cells such that the desired protein is expressed and recovering the protein from the culture.

By way of example, the nucleotide sequence in SEQ ID NO: 1 is inserted downstream of the SV40 promoter in the pGEX plasmid vector, and either transiently or

stably expressed in COS -7 cells. Expression of the protein according to the invention can be detected following disruption of the cells by Western blotting .

It may be desirable to produce the individual functional domains of the protein according to the invention in isolation from the rest of the molecule. This may be achieved using the above standard recombination DNA techniques except that in this instance the DNA sequence used is that encoding one of the partial amino acid sequences of the domains identified in Figure 1 or a combination of these.

By way of further example, the nucleotide sequence in SEQ ID NO: 1 is inserted downstream of the SV40 promoter and the glutathione-S-transferase (GST) coding sequence in the pBC plasmid vector, and either transiently or stably expressed in COS -7 cells allowing expression of the corresponding fusion protein. Expression of the fusion protein can be detected following disruption of the cells by Western blotting with antibodies to GST, and furthermore the fusion protein can be used in an affinity binding procedure to find proteins which are functional partners of the protein of the invention from cell extracts.

A ZGGBP1 protein of the invention may in particular be used to screen for compounds which regulate the activity of the enzymes and the invention extends to such a screen and to the use of compounds obtainable therefrom to regulate the activity of the protein in vivo.

Thus according to a further aspect of the invention we provide a method for identifying a compound capable of modulating the action of a ZGGBP1 protein which method comprises subjecting one or more test compounds to a screen comprising (A) a protein containing the amino acid sequence shown in SEQ ID NO: 2 or a homologue or fragment thereof, or (B) the nucleotide sequence shown in SEQ ID NO: 1 or a homologue or fragment thereof, or (C) a host cell expressing a ZGGBP1 polypeptide or a homologue or fragment thereof.

The screen according to the invention may be operated using conventional procedures, for example by bringing the test compound or compounds to be screened and an appropriate substrate into contact with the protein or a cell capable of producing it and determining affinity for the protein in accordance with conventional procedures.

Any compound identified in this way may be used in the treatment of humans and/or other animals of one or more of the above mentioned diseases. The invention thus

extends to a compound selected through its ability to regulate the activity of the protein in vivo as primarily determined in a screening assay utilising the protein containing an amino acid sequence shown in SEQ ID NO: 2 or a homologue or fragment thereof, or a gene coding therefor for use in the treatment of a disease in which the over- or under-activity or unregulated activity of the protein is implicated.

In a further aspect of the invention we provide examples of insertions/deletions and single base change polymorphisms (mutations) as outlined in Figure 6, 7, 8, 9 and 10.

The ZGGBP1 gene of the invention may also be used as the basis for diagnosis, for example to determine expression levels in a human subject, by for example direct DNA sequence comparison or DNA/RNA hybridisation assays. Diagnostic assays may involve the use of nucleic acid amplification technology such as the PCR and in particular the Amplification Refractory Mutation System (ARMS) as claimed in our European Patent No. 0 332 435. Such assays may be used to determine allelic variants of the gene, for example insertions, deletions and/or mutations such as one or more point mutations. Such variants may be heterozygous or homozygous.

In a further aspect of the invention, amplification primers may be provided for use in the above diagnostic methods. In general, these are provided as a set and used for PCR amplification. One of the primers conveniently hybridises to a ZGGBP1 locus outside the region defined by base pairs 516-3568 thus allowing the ZGGBP1 gene on 18q21 to be identified to the exclusion of other loci.

The ZGGBP1 gene may also be used in gene therapy, for example where it is desired to modify the production of the protein in vivo, and the invention extends to such uses.

Knowledge of the gene according to the invention also provides the ability to regulate its expression in vivo by for example the use of antisense DNA or RNA. Thus, according to a further aspect of the invention we provide an antisense DNA or an antisense RNA which is complementary to the polynucleotide sequence shown in SEQ ID NO: 1. By complementary we mean that the two molecules can base pair to form a double stranded molecule.

The antisense DNA or RNA for co-operation with the gene in SEQ ID NO: 1 can be produced using conventional means, by standard molecular biology and/or by chemical synthesis as described above. If desired, the antisense DNA or antisense RNA may be

chemically modified so as to prevent degradation in vivo or to facilitate passage through a cell membrane and/or a substance capable of inactivating mRNA, for example ribozyme, may be linked thereto and the invention extends to such constructs.

The antisense DNA or antisense RNA may be of use in the treatment of diseases or disorders in humans in which the over- or under-regulated production of the gene product has been implicated. Such diseases or disorders may include those described under the general headings of neurologic, eg.stroke, dementia, renal eg. hypertension, nephrosis, cardiovascular disorders.

Convenient DNA sequences may be obtained using conventional molecular biology procedures, for example by probing a human genomic or cDNA library with one or more labelled oligonucleotide probes containing 10 or more contiguous nucleotides designed using the nucleotide sequences described here. Alternatively, pairs of oligonucleotides one of which is homologous to the sense strand and one to the antisense strand, designed using the nucleotide sequences described here to flank a specific region of DNA may be used to amplify that DNA from a cDNA library.

The ZGGBP1 protein of the invention and homologues or fragments thereof may be used to generate substances which selectively bind to it and in so doing regulate the activity of the protein. Such substances include, for example, antibodies, and the invention extends in particular to an antibody which is capable of recognising one or more epitopes containing the protein binding domains shown in Figure 1. In particular the antibody may be neutralising antibody.

As used herein the term antibody is to be understood to mean a whole antibody or a fragment thereof, for example a F(ab)2, Fab, FV,. VH or VK fragment, a single chain antibody, a multimeric monospecific antibody or fragment thereof, or a bi- or multi-specific antibody or fragment thereof.

The invention will now be illustrated but not limited by reference to the following detailed description, References, Examples and Figures wherein:

Figure 1 shows the predicted amino acid sequence of ZGGBP1. The C2 domain is indicated by carets , the four WW domains are indicated by asterisks and the Hect domain is indicated by underlining .

Figure 2 shows a comparison of amino acid sequences of human ned4 Swissprot entry P46934 and ZGGBP1.

Figure 3 shows a Northern blot analysis of various human tissues probed with ZGGBP1.

Figure 4 shows a comparison of the nucleic acid sequences of human and mouse ZZGBP1. The mouse sequence is a partial cDNA which spans the C-terminal portion of the human protein coding region.

Figure 5 shows a comparison of the nucleic acid sequences for ZGGBP1 and Pub3

Figure 6 shows a polymorphism located at position 3554 of the cDNA sequence

Figure 7 shows a polymorphism located at position 4828 of the cDNA sequence

Figure 8 shows a polymorphism located in an intronic sequence derived from a BAC containing ZGGBP1

Figure 9 shows a variable number of tetranucleotide repeats located within an intronic sequence from ZGGBP1

Figure 10 shows an insertion at position 4032 of the cDNA sequence

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Example 1

Identification of ZGGBP1

We used two methods for investigating the 18q21 region of interest. In one method we used positional cloning to identify novel transcripts from physical clones representing the region and in a second method we utilised public databases to identify transcripts which had been assigned to a low resolution map of the region by radiation hybrid mapping and assigned them to physical clones representing a high resolution map of the region.

Method 1 - Positional Cloning

The 18q21 region described by Stine et al. (1995) is delimited by the STS markers used by that group to identify linkage. They found the most strongly linked marker to be D18S41, which had a LOD score of 3.51 in cases of paternal inheritance. Linkage declined over flanking markers. We identified a set of four Yeast Artificial Chromosomes (YACs) which comprised a contiguous overlapping set of genomic clones covering the defined region by the presence in those YACs of STS markers used in the Stine study.

DNA from the YACs was prepared and used in a PCR-based hybridisation approach to enrich for transcripts from a human fetal brain cDNA library. This approach, known as direct selection (Lovett et al. 1991) has been shown to be efficient in identifying transcripts present on large genomic clones.

Method 2 - Refining Radiation Hybrid Mapped Transcripts

The UNIGENE database is a repository for transcripts which have been mapped by taking representative Expressed Sequence Tagged Sites (ESTs) and performing PCR analysis on a panel of radiation hybrids which have been calibrated with respect to a framework of 1000 genetic markers (Schuler et al. 1996). We found 36 EST clusters which had been mapped to a radiation hybrid map interval which corresponded to the 18q21 region of interest and to flanking regions outside.

All the ESTs were tested by PCR on our YAC genomic clones to determine which were present. We found approximately half of the ESTs to be present within the genomic clones and were able to order them based on their position within the YAC contig.

Results

Several clones from our direct selection experiments showed sequence homology to a known EST which we had previously shown to be present in two of the YACs within the contig. The EST was representative of a cluster of sequences. All of these sequences were assembled together using DNAStar Seqman and the consensus sequences obtained were used iteratively to search for other database members within both Unigene, dbEST and EMBL databases. This resulted in the surprising identification of two further clusters of ESTs which had previously not been related to each other on the basis of sequence analysis. The two new EST clusters were annotated as having sequence similarity to ned-4. This was an unexpected finding since we had recently mapped the human ned-4 by Fluorescence In Situ Hybridisation (FISH) to chromosome 15. We were aware that ned-4 was involved in neuronal cell signalling and we concluded that the EST cluster on 18q21 must represent a closely related gene and therefore likely to be involved in affective neurological disorders such as bipolar affective disorder.

The assembly of the EST clusters did not give rise to a single complete contiguous sequence. The reason for this is that many of the EST sequences were derived from IMAGE cDNA clones for which end sequence only was available. In order to fill in the gaps and give a complete contig, four of these clones (IMAGE I.D. 80951, 33059, 79526 and 79984) were sequenced completely to fill the gaps and give an entire complete contiguous sequence. Comparison of the sequence with ned-4 showed that the contig comprised 2kb of 3' Untranslated Region (UTR) and 700bp of the coding region of a gene

which had approximately 85% identity at the amino acid level to ned-4 and which we named ZGGBP1.

Isolation of the full length gene for ZGGBP1

The extending of partial transcripts to full length clones can be a complex and difficult process requiring skill and expertise for success. Having considered several possibilities, we opted for a PCR-based approach to isolate and characterise the full length ZGGBP1 gene. Human foetal brain double stranded cDNA was synthesised from mRNA using standard methods (Sambrook et al. 1989) and ligated into lambda Zap vector by use of adapters. However, in order to minimise the loss of transcripts often seen following the cloning step, the resulting ligation mix was not cloned but was instead used as a template for PCR. Oligonucleotide primers specific to ZGGBP1 were used in combination with vector specific primers to amplify DNA across the unknown part of the gene. Since the distance to be covered was unknown, we performed long PCR using the commercially available BCL Expand enzyme and long (30mer) oligonucleotide primers. Since we were using unamplified material, where our target cDNAs were likely to be present only in very small amounts, we utilised a secondary PCR step with nested oligonucleotide primers and again using long PCR to yield sufficient PCR products to be visible by gel analysis and also to minimise the possibility of non-specific PCR amplification. The PCR products derived from these experiments were then purified and sequenced directly. Where necessary, the DNA sequence obtained was used to design further primers to walk along the gene in a 3' - 5' direction. The complete nucleotide sequence derived from this work is 5.2kb and the translated amino acid sequence is shown in SEQ ID NO: 1.

The amino acid sequence derived from the cDNA was compared with that of ned-4 and is shown in Figure 2. The proteins diverge markedly towards the N-terminal portion of the protein, although there is conservation of the common functional motifs.

Northern analysis using a probe derived from the 3'UTR of ZGGBP1 showed a band at approximately 4.8kb but also a more abundant band of 9kb in size in several neurological tissues, with the exception of medulla or spinal cord. These bands are likely to be due to alternative splicing (Figure 3). Other tissues contained the 4.8kb band at higher abundance with respect to the 9kb band and also a 4kb band. ZGGBP1 was

expressed in all tissues examined with the exception of liver where we could not detect a transcript at our current detection sensitivity.

Comparison of Amino Acid Sequences of human ned-4 and ZGGBP1

A comparison of the amino acid sequences of human ned-4 and ZGGBP1 is shown in Figure 6. The two proteins have a high level of homology over much of the C-terminal region, including the Hect and WW domains, but diverge over the central portion of the protein. There is a further block of homology near to the N-terminal region, including the C2 domain. The presence of these domains in ZGGBP1 suggests some common functionality with ned-4.

Identification of polymorphic variants of ZGGBP1

500bp regions of the ZGGBP1 cDNA were PCR amplified from a variety of tissues and lymphoblastoid cell lines. Sequencing was carried out and polymorphisms identified as outlined in Figures 5 and 6. Some intronic sequence had been identified from a genomic clone and sequence analysis of these regions identified a further polymorphic variant as outlined in Figure 7. A tetranucleotide repeat (GATT) was also identified in an intronic sequence derived from this BAC and this was found to have variable numbers of repeats (Figure 8).

Isolation of Genomic Clone for ZGGBP1

The Research Genetics human Bacterial Artificial Chromosome (BAC) library (Shizua et al. 1992, Kim et al. 1996) was screened by PCR using primers specific to the 3'UTR of ZGGBP1 and BACs were isolated. These are being used to characterise the structural gene including the intron/exon structure and the 5' regulatory region.

Isolation of Mouse homologue for ZGGBP1

The full length sequence of ZGGBP1 shown in SEQ ID NO: 1 was used to search the dbEST database to identify homologous mouse sequences. Three overlapping IMAGE clones were identified (IMAGE I.D.479436, 573510, 482922) comprising a partial transcript. Comparison of the mouse and human nucleotide sequence is shown in Figure 4. The mouse clones were isolated for use as a probe for in situ hybridisation on sections

of mouse brain during development, and as a probe of mouse genomic libraries to isolate genomic clones and to produce transgenic mice by gene targeting using homologous recombination.

CLAIMS

1. A polynucleotide comprising a nucleic acid sequence which encodes the polypeptide of Seq ID No 2, and homologues and fragments thereof.

2. A polynucleotide as claimed in claim 1 which comprises the cDNA sequence of Seq ID No 1.

3. Polymorphic variants of the polynucleotide as claimed in claim 2, selected from the group in which:

i) T at position 3554 is replaced by C.

ii) C at position 4828 is replaced by G.

iii) T within an intronic region associated with ZGGBP1 is replaced by C.

iv) C is inserted at position 4032.

4. A polynucleotide which comprises an animal homologue of the nucleic acid claimed in claims 1-3.

5. A polynucleotide as claimed in claim 4 which comprises the cDNA sequence of Seq ID No 3, and homologues and fragments thereof.

6. A polynucleotide which is capable of specifically hybridising to eight or more contiguous nucleotides comprised in Seq ID No 1 or Seq ID No 3 or comprised in the complementary strands thereof.

7. A polynucleotide which comprises a ZGGBP1 gene fragment.

8. A vector comprising a polynucleotide of claims 1-7.

9. A host cell transformed with a vector of claim 8.

10. A polypeptide comprising the amino acid sequence of Seq ID No 2 and homologues and fragments thereof.

11. A polypeptide comprising the amino acid sequence of Seq ID No 4 and homologues and fragments thereof.

12. A fusion protein in which a polypeptide of claim 10 or claim 11 is fused with glutathione-S-transferase.

13. A method for producing cells which express a polypeptide of claim 10 or claim 11 or a fusion protein of claim 12, comprising:

a) culturing a host cell of claim 9 under conditions suitable for the expression of the polypeptide.

b) recovering the polypeptide from the host cell culture.

14. A method for identifying a compound capable of modulating the activity of a ZGGBP1 protein , which method comprises subjecting one or more test compounds to a screen comprising:

a) a protein as claimed in claims 10-12 or a homologue or fragment thereof,

or

b) a polynucleotide as claimed in claims 1-7 or a homologue or fragment thereof,

or

c) a host-cell expressing a polypeptide of a ZGGBP1 molecule,

and measuring an effect of the test compound on ZGGBP1 activity.

15. A compound that modulates the activity of a human ZGGBP1 identified by the method of claim 14.

16. A pharmaceutical composition comprising a compound that modulates the activity of a protein identified by the method of claim 14.

17. A diagnostic assay for the detection of ZGGBP1, which assay comprises measuring the presence or absence of a protein as claimed in claims 10-12 or a polynucleotide as claimed in claims 1-7.

18. An antisense molecule comprising a complement of the polynucleotide in claims 1-7 or a biologically effective fragment thereof.

19. Use of a polynucleotide as claimed in claims 1-7 or claim 18 in gene therapy.

20. An antibody specific for a protein of claims 10-12 or fragments thereof.

21. A set of amplification primers for selective amplification of a ZGGBP1 gene sequence.

FIGURE 1

MFRLRSWASSTTGSRYGSAFCGSPTLAWCVCVPVCYGESRILRVKVVSG

IDLAKKDIFGASDPYVKLSLYVADENRELALVQTKTIKKTLNPKWNEEF
^^

YFRVNPSNHRLLFEVFDENRLTRDDFLGQVDVPLSHLPTEDPTMERPYT
^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^

FKDFLLRPRSHKSRVKGFLRLKMAYMPKNGGQDEENSDQRDDMEHGWEV

VDSNDSASQHQEELPPPPLPPGWEEKVDNLGRTYYVNHNNRTTQWHRPS
**********^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^

LMDVSSESDNNIRQINQEAAHRRFRSRRHISEDLEPEPSEGGDVPEPWE
*
TISEEVNIAGDSLGVVLPPPPASPGSRTSPQELSEELSRRLQITPDSNG

EQFSSLIQREPSSRLRSCSVTDAVAEQGHLPPPSVAYVHTTPGLPSGWE
^^
****

ERKDAKGRTYYVNHNNRTTTWTRPIMQLAEDGASGSATNSNNHLIEPQI
**********^^
****
RRPRSLSSPTVTLXAPLEGAKDSPVRRAVKDTLSNPQSPQPSPYNSPKP

QHKVTQSFLPPGWEMRIAPNGRPFFIDHNTKTTTWEDPRLKFPVHMRSK

TSLNPNDLGPLPPGWEERIHLDGRTFYIDHNSKITQWEDPRLQNPAITG
^^
****

PAVPYSREFKQKYDYFRKKLKKPADIPNRFEMKLHRNNIFEESYRRIMS

VKRPDVLKARLWIEFESEKGLDYGGVAREWFFLLSKEMFNPYYGLFEYS

ATDNYTLQINPNSGLCNEDHLSYFTFIGRVAGLAVFHGKLLDGFFIRPF

YKMMLGKQITLNDMESVDSEYYNSLKWILENDPTELDLMFCIDEENFGQ

TYQVDLKPNGSEIMVTNENKREYIDLVIQWRFVNRVQKQMNAFLEGFTE

LLPIDLIKIFDENELELLMCGLGDVDVNDWRQHSIYKNGYCPNHPVIQW

FWKAVLLMDAEKRIRLLQFVTGTSRVPMNGFAELYGSNGPQLFTIEQWG

SPEKLPRAHTCFNRLDLPPYETFEDLREKLLMAVENAQGFEGVD.

FIGURE 2

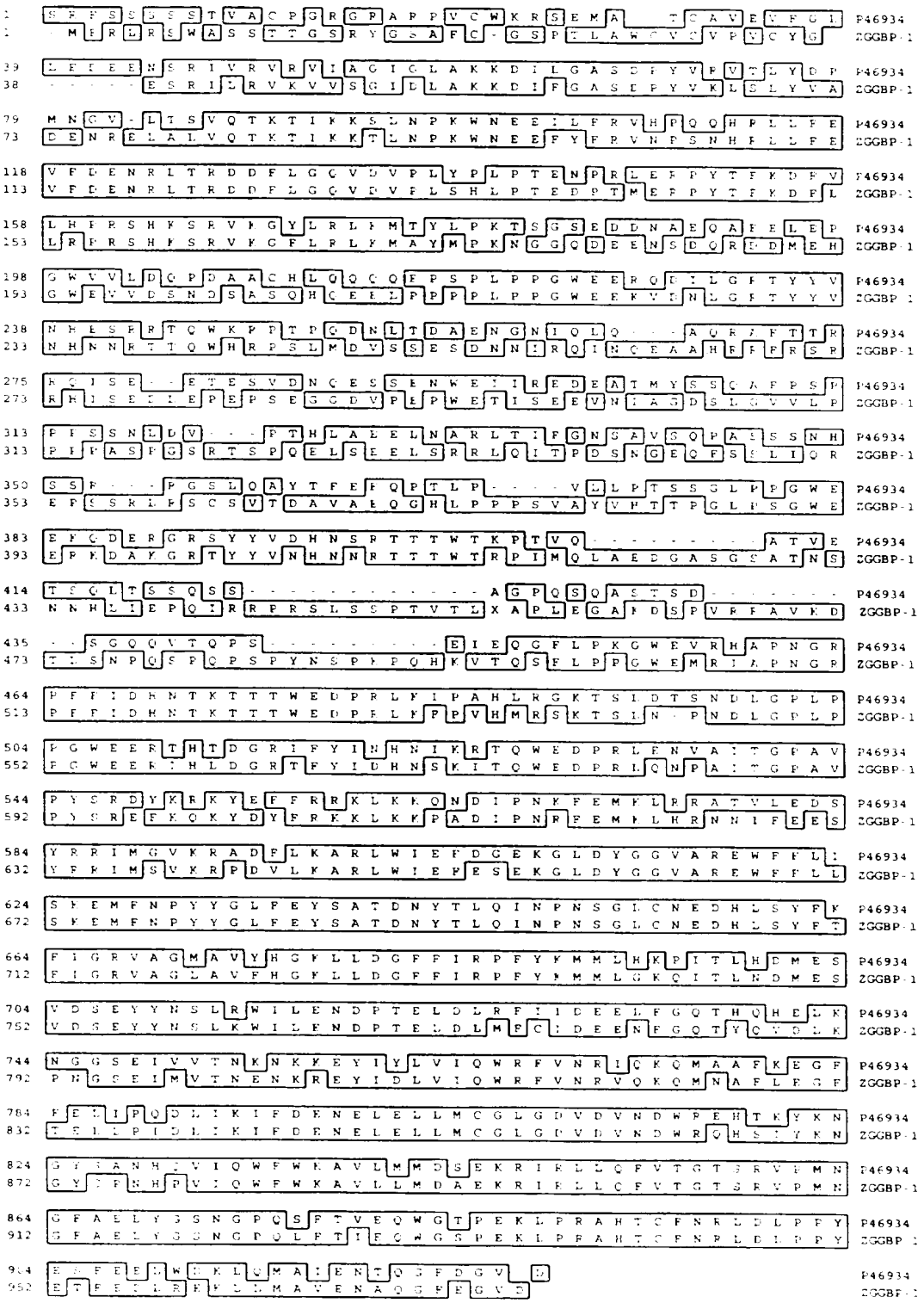

FIGURE 3

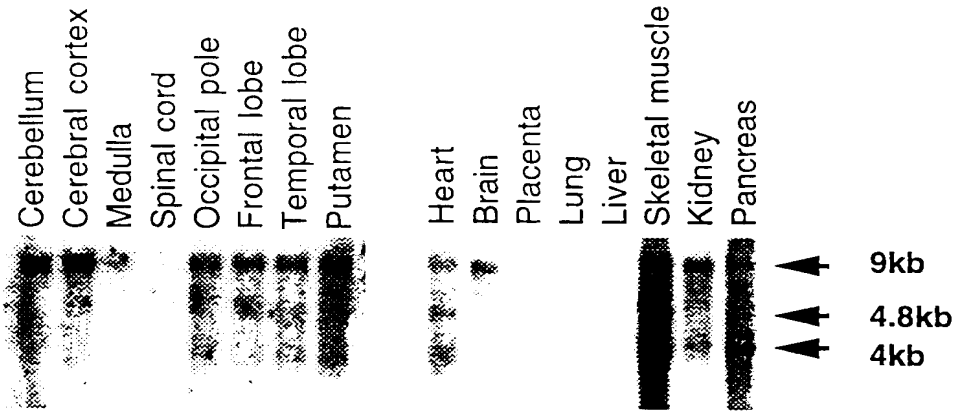
Cerebellum
Cerebral cortex
Medulla
Spinal cord
Occipital pole
Frontal lobe
Temporal lobe
Putamen
Heart
Brain
Placenta
Lung
Liver
Skeletal muscle
Kidney
Pancreas
9kb
4.8kb
4kb

FIGURE 4

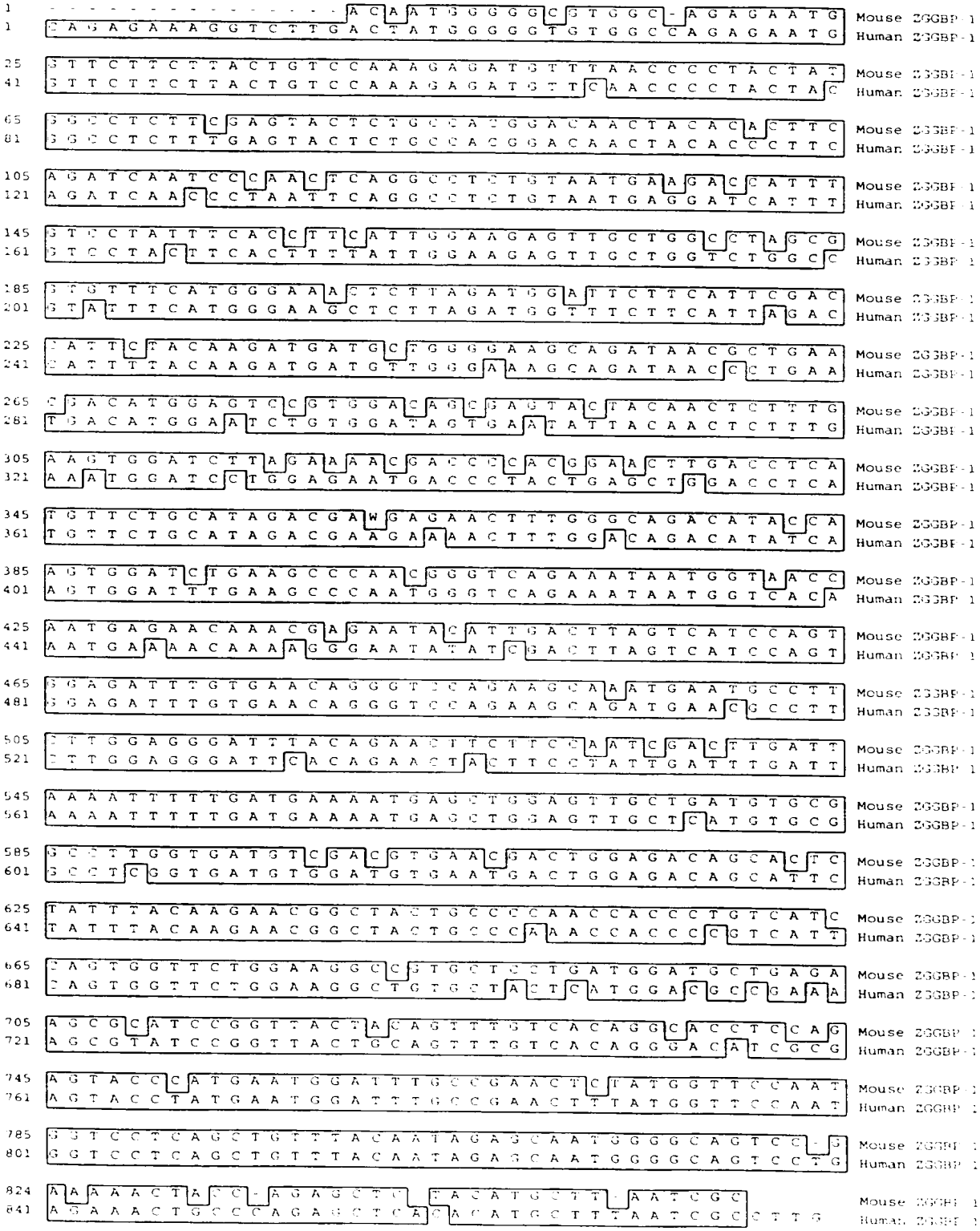

FIGURE 5

```
1    CGG---------TA----TCAGCAGAGG------------  Pub-3.seq
1    CAAGCGCGCAATTAACCCTCACTAAAGGAACACCAACAC   ZGGBP1.seq

16   -----------TGTGT--------------ACGGGCACTG  Pub-3.seq
41   GTCGCCAGGACTGCGCCGTTCGCTGCGCTCATAGGCGGCG  ZGGBP1.seq

31   CTTT----AAAACTGGAAGGA------GG-----------  Pub-3.seq
81   ATTTCATCAAGGGTGGCAAGGATCGCCTGGTCGACGGTCA  ZGGBP1.seq

51   --------------------------------------AA  Pub-3.seq
121  GGTCGTCCTCGACGCGGTTGCCCTCCTCGTCCTGTTCCAG  ZGGBP1.seq

53   GACGAG-------------------GCCAGGGAG------  Pub-3.seq
161  GGTGAGTGGGCGATACCAGGTGTCCACCGGGAAGGTACGG  ZGGBP1.seq

68   ---------------------------CCGGAG-------  Pub-3.seq
201  CCCGACACCTCGACAATCGGCGCATCGTCGAAGTGCTTGG  ZGGBP1.seq

74   ------------GGTCA-------CCAGGTA-----GAT   Pub-3.seq
241  AAAAGCGCTCCAGGTCGATGGTGGCCGAGGTGATGATGAC  ZGGBP1.seq

90   TTCCAG------CAGCGC-------------------TAG  Pub-3.seq
281  TTTCAGGTCGGGGCGACGCGGCAACAGGGTCTTGAGGTAG  ZGGBP1.seq

105  TCCAGCTGAA---CACTTTCCAG--------------CCT  Pub-3.seq
321  CCGAGCAGGAAGTCGATGTTCAGGCTGCGTTCGTGGGCTT  ZGGBP1.seq

128  TGTT---------------------------TTTCAGCAG  Pub-3.seq
361  CGTCGACGACAGGCTCGCGTTATGGCTCCGCTTTCTGCGG  ZGGBP1.seq

141  CTTT------------------------------------  Pub-3.seq
401  CTCTCCTACCCTGGCATGGTGTGTGTGTGTGCCTGTGTGC  ZGGBP1.seq

145  ----------------------GAGGAAAAGT---AT---  Pub-3.seq
441  TACGGAGAGTCCCGTATTCTCAGAGTAAAAGTTGTYTCTG  ZGGBP1.seq
```

FIGURE 5 continued

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157  -----------------------------------AGTGA  Pub-3.seq
481  GAATWGATCTCGCCAAAAAGGACATCTTTGGAGCCAGTGA  ZGGBP1.seq

162  TCCGTATGTGAAACTTTCATTGTACGTAGCGGATGAGAAT  Pub-3.seq
521  TCCGTATGTGAAACTTTCATTGTACGTAGCGGATGAGAAT  ZGGBP1.seq

202  AGAGAACTTGCTTTGGTCCAGACAAAAACAATTAAAAAGA  Pub-3.seq
561  AGAGAACTTGCTTTGGTCCAGACAAAAACAATTAAAAAGA  ZGGBP1.seq

242  CACTGAACCCAAAATGGAATGAAGAATTTTATTTCAGGGT  Pub-3.seq
601  CACTGAACCCAAAATGGAATGAAGAATTTTATTTCAGGGT  ZGGBP1.seq

282  AAACCCATCTAATCACAGACTCCTATTTGAAGTATTTGAC  Pub-3.seq
641  AAACCCATCTAATCACAGACTCCTATTTGAAGTATTTGAC  ZGGBP1.seq

322  GAAAATAGACTGACACGAGACGGCTTCCTGGGCCAGGTGG  Pub-3.seq
681  GAAAATAGACTGACACGAGACGACTTCCTGGGCCAGGTGG  ZGGBP1.seq

362  ACGTGCCCCTTAGTCACCTTCCGACAGAAGATCCAACCAT  Pub-3.seq
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761  GGAGCGACCCTATACATTTAAGGACTTTCTCCTCAGACCA  ZGGBP1.seq

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841  AAATGGCCTATATGCCAAAAAAATGGAGGTCAAGATGAAGA  ZGGBP1.seq

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FIGURE 5 continued

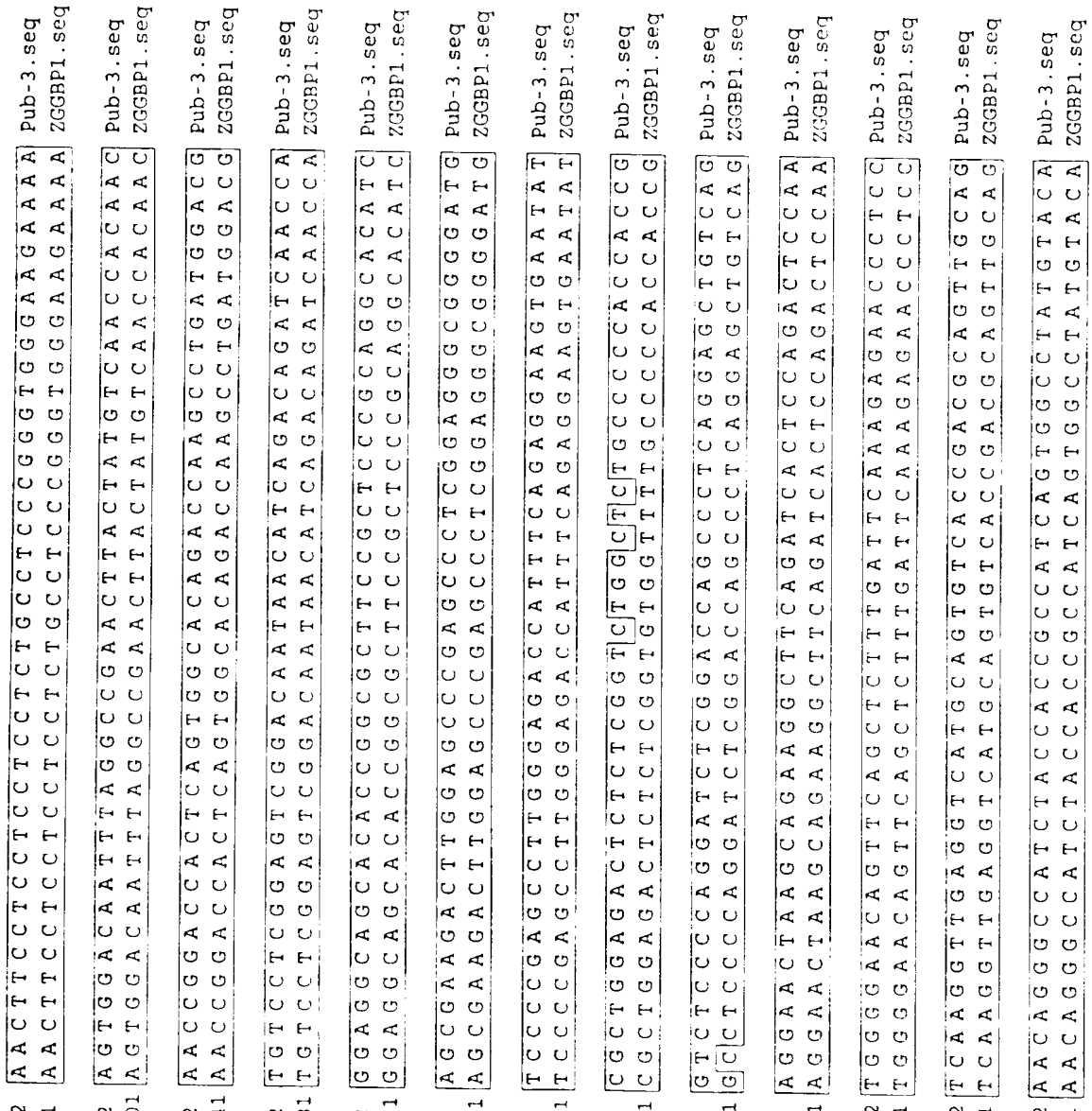

FIGURE 5 continued

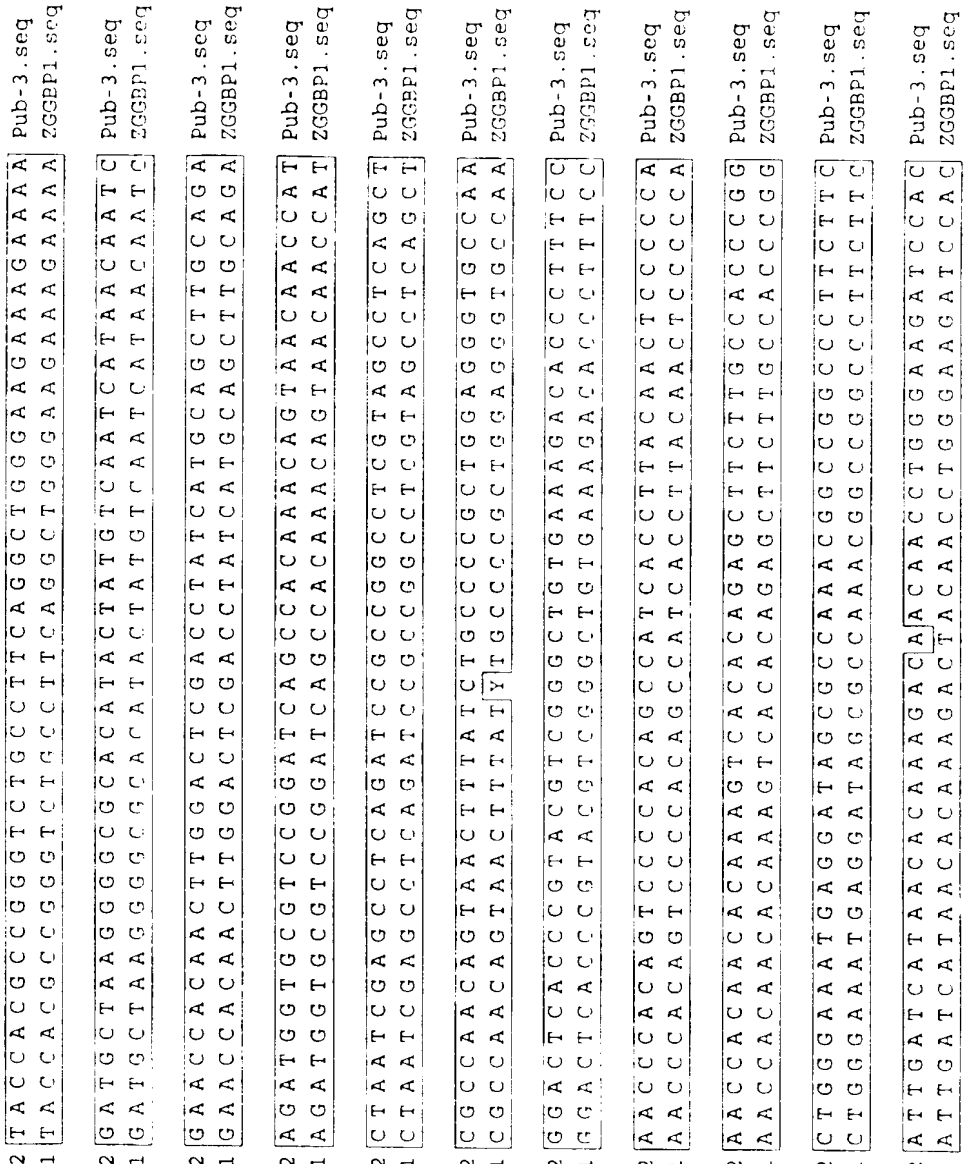

FIGURE 5 continued

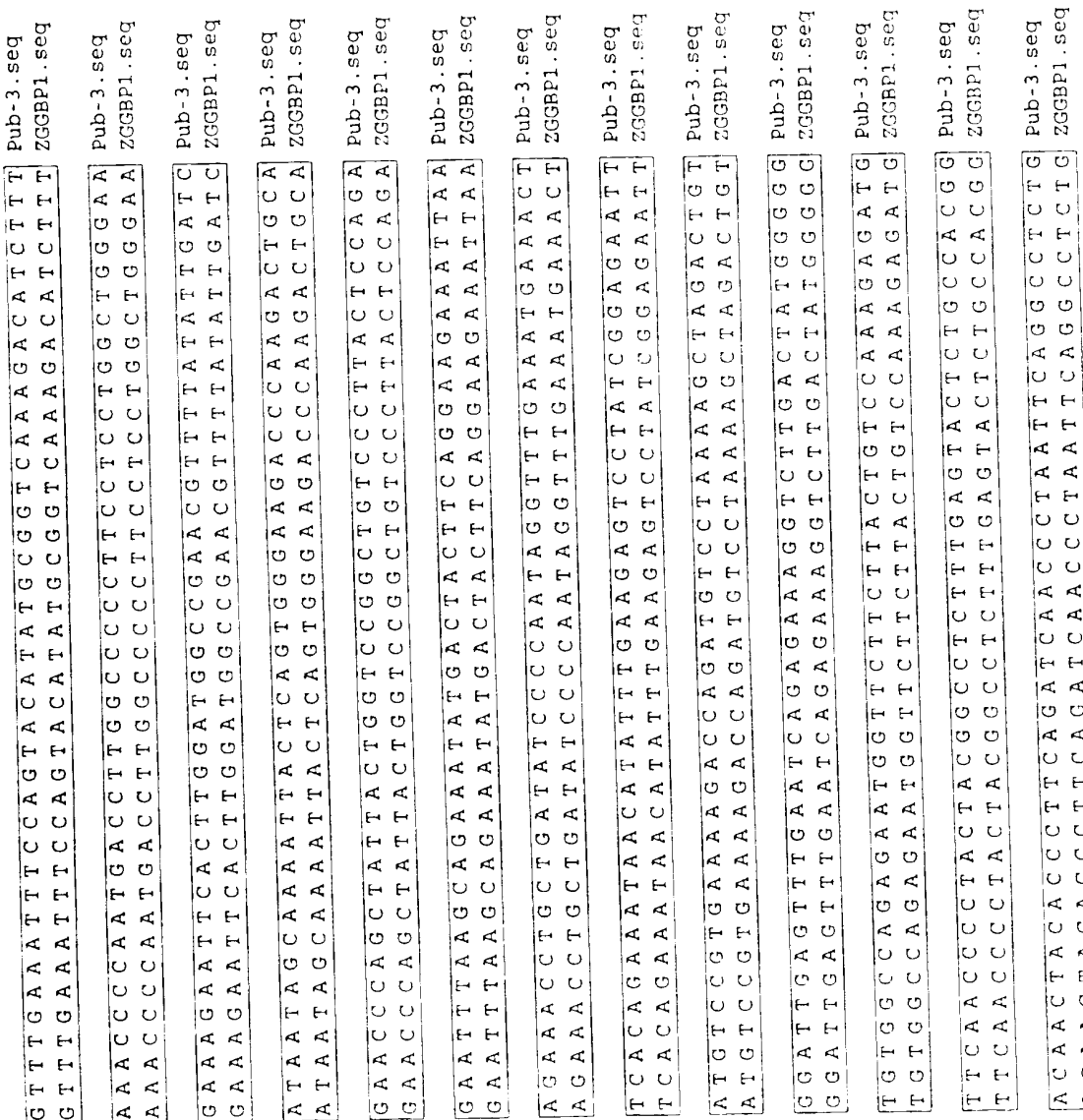

FIGURE 5 continued

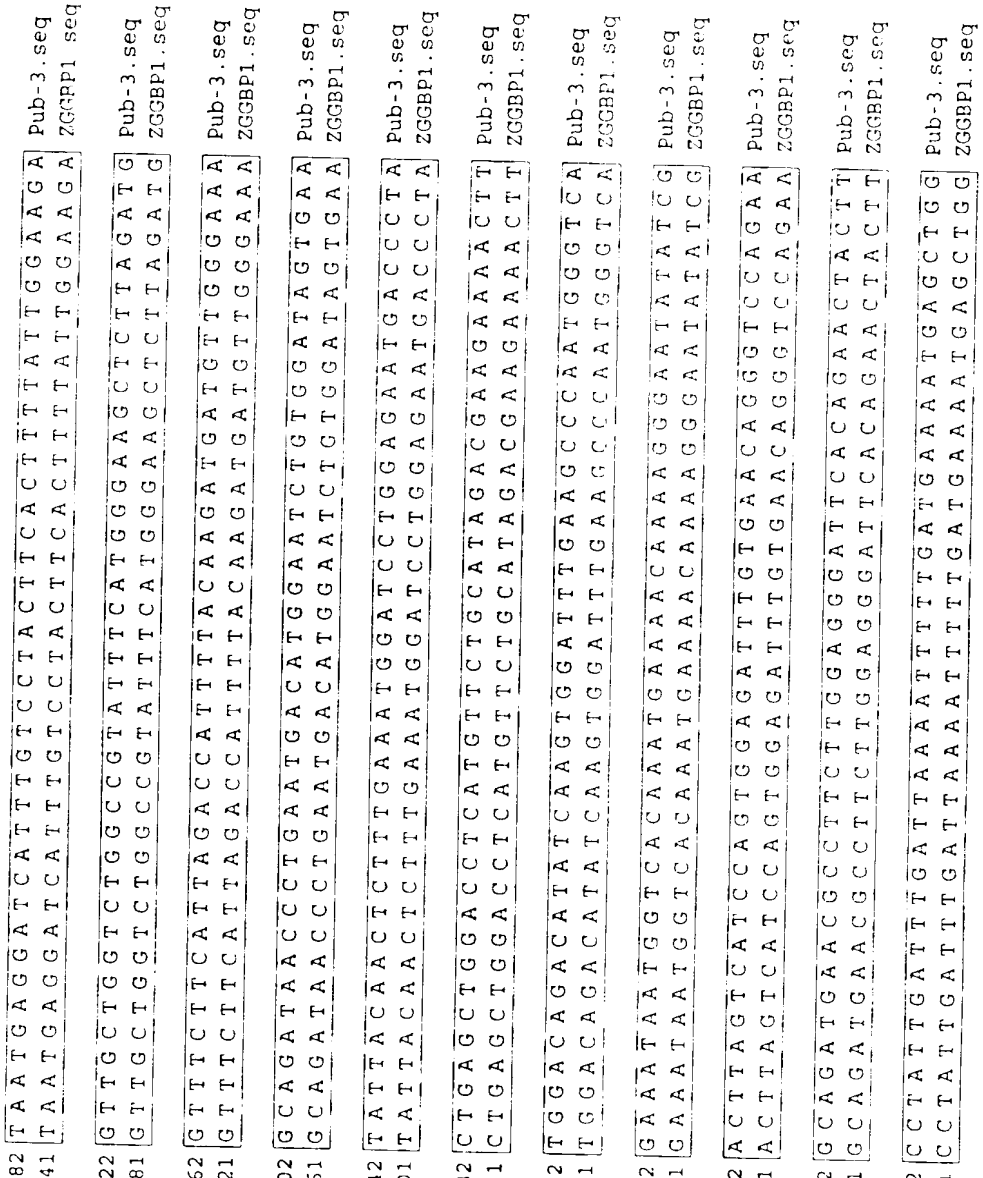

FIGURE 5 continued

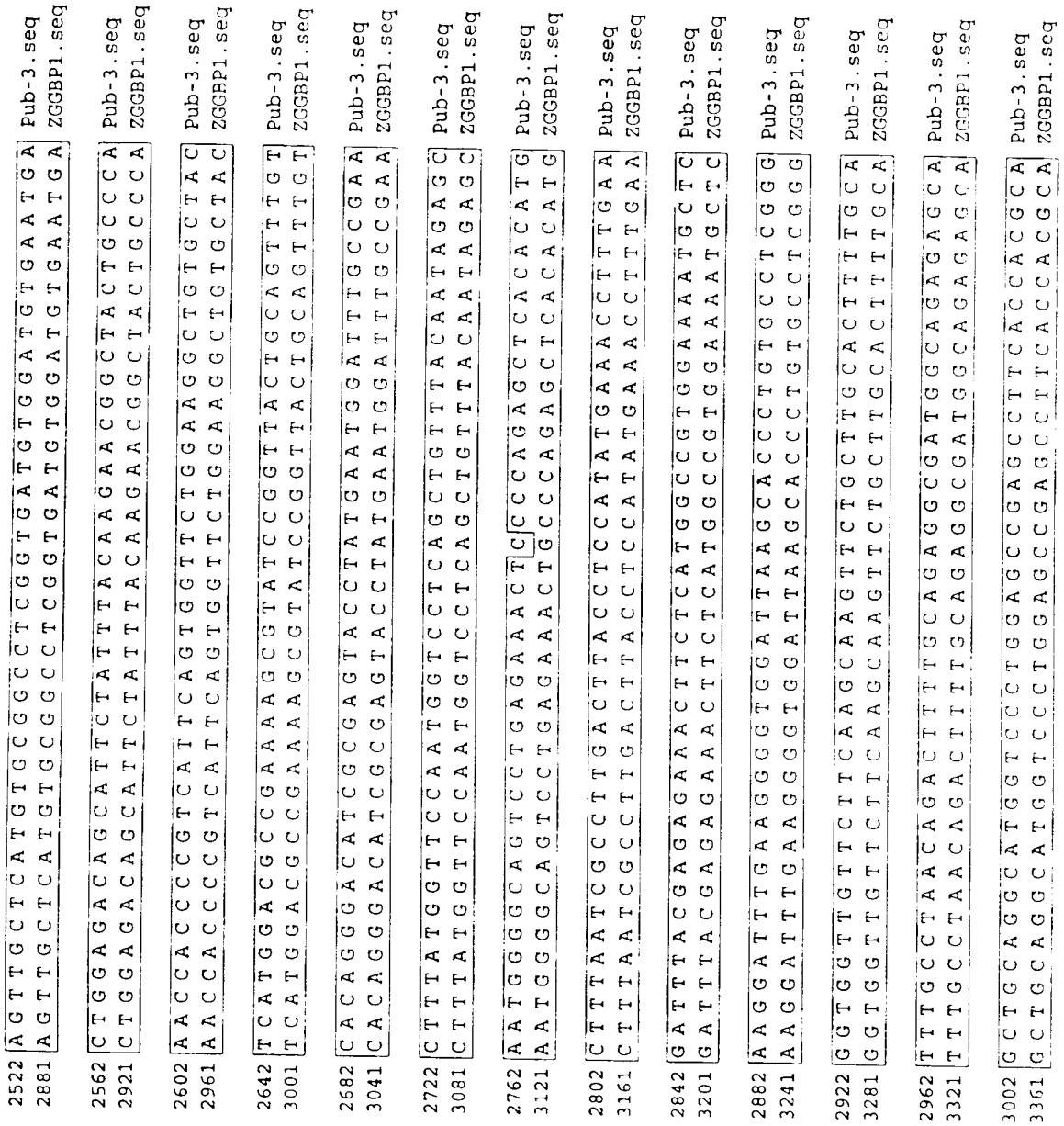

FIGURE 5 continued

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3208 -----------------------------AAAAAA----- Pub-3.seq
3880 ATAATGATAATTTTTTCCATACTCAGAATGAAAAACTGGA ZGGBP1.seq

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3214 ---------------------------------------- Pub-3.seq
3960 CTAATAGCTACAGGCTGAGAGAATTGTAACATAGCATGAC ZGGBP1.seq

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3214 ---------------------------------------- Pub-3.seq
4160 TTGCTGTTTGTAGTAGAGACATTTTGAATGAAACTTGGCA ZGGBP1.seq

3214 ---------------------------------------- Pub-3.seq
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3214 ---------------------------------------- Pub-3.seq
4240 TCCTGTTTGTATGCGTTTGCTAATGGTAGCTAAATAACCA ZGGBP1.seq

3214 ---------------------------------------- Pub-3.seq
4280 GTTTTTGTTGTAAATGCACCAATTCTGAAGGCACTTTATG ZGGBP1.seq

3214 ---------------------------------------- Pub-3.seq
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FIGURE 5 continued

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3214 ---------------------------------------- Pub-3.seq
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3214 ---------------------------------------- Pub-3.seq
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3214 ---------------------------------------- Pub-3.seq
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3214 ---------------------------------------- Pub-3.seq
4720 TGATTCTTTTCCCACGTTGTAAGTCTCCATTGCAGAATTG ZGGBP1.seq

3214 ---------------------------------------- Pub-3.seq
4760 TCGTGCGTTTGAGAAAACACCTGAGGCAGTGTGGGAGTTG ZGGBP1.seq
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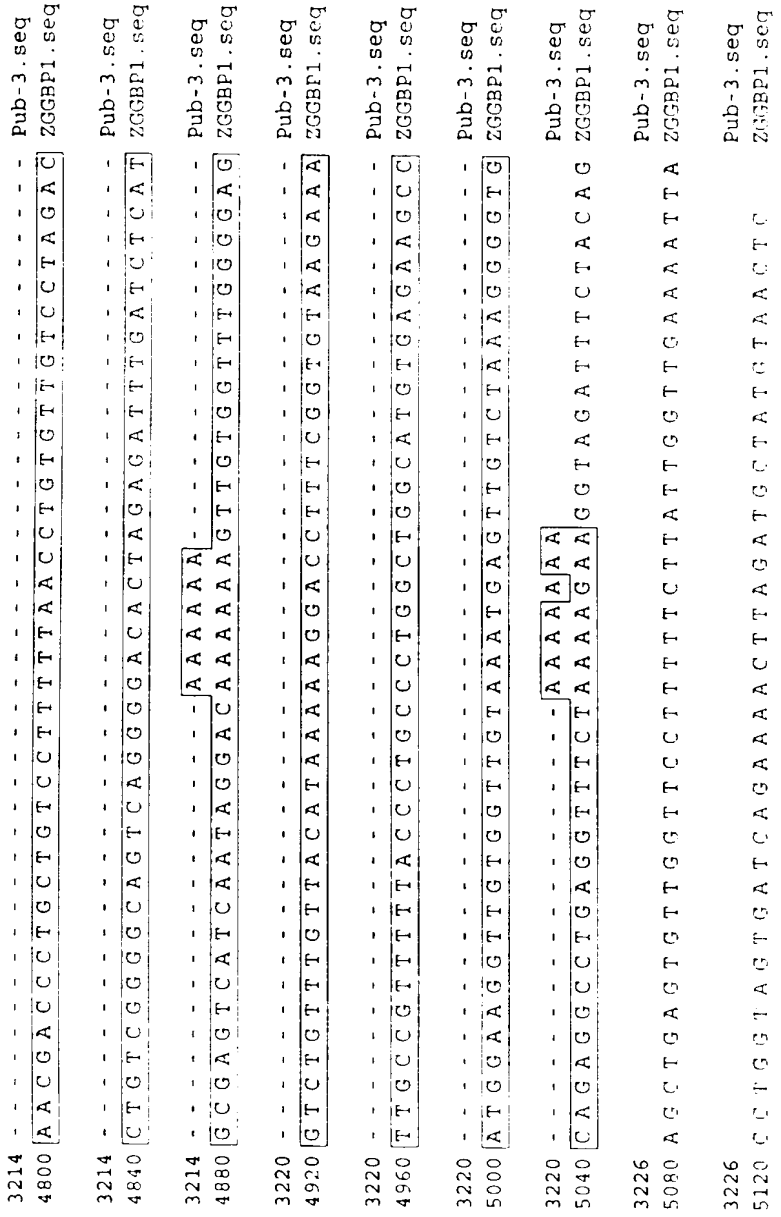
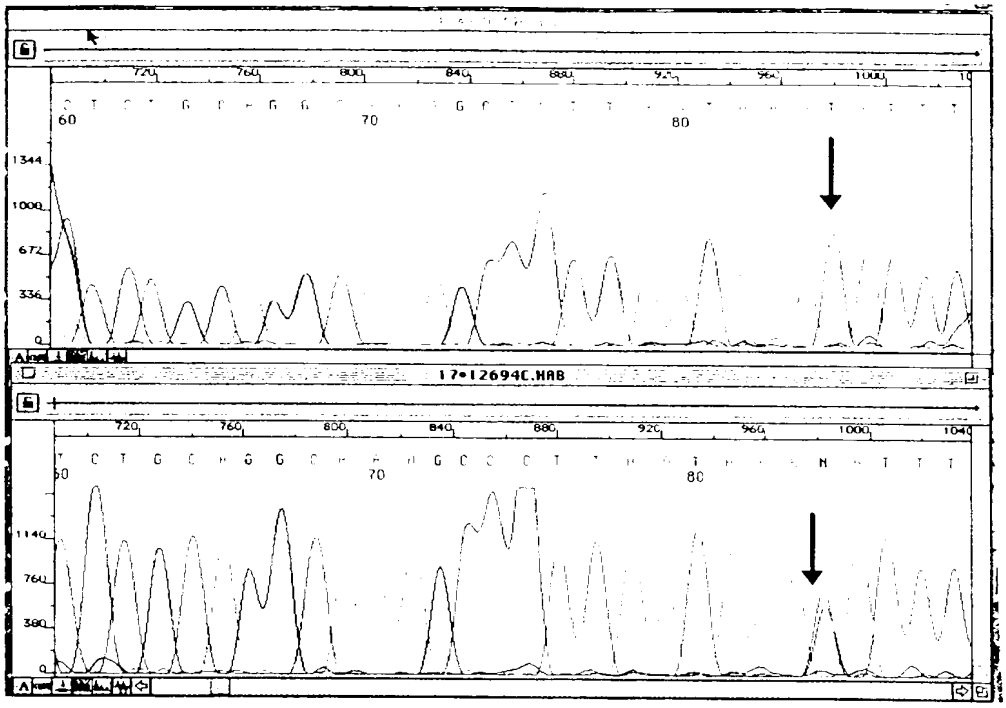
FIGURE 5 continued

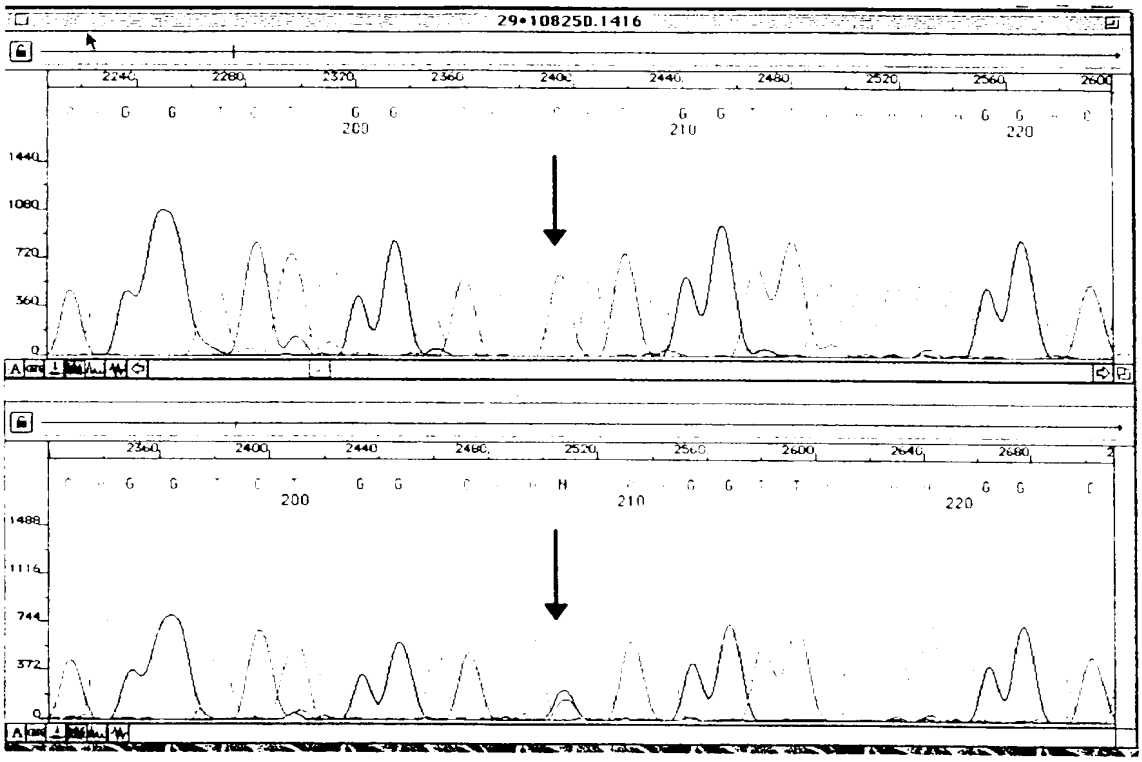


Decoration '1': Box residues that match the Consensus exactly.



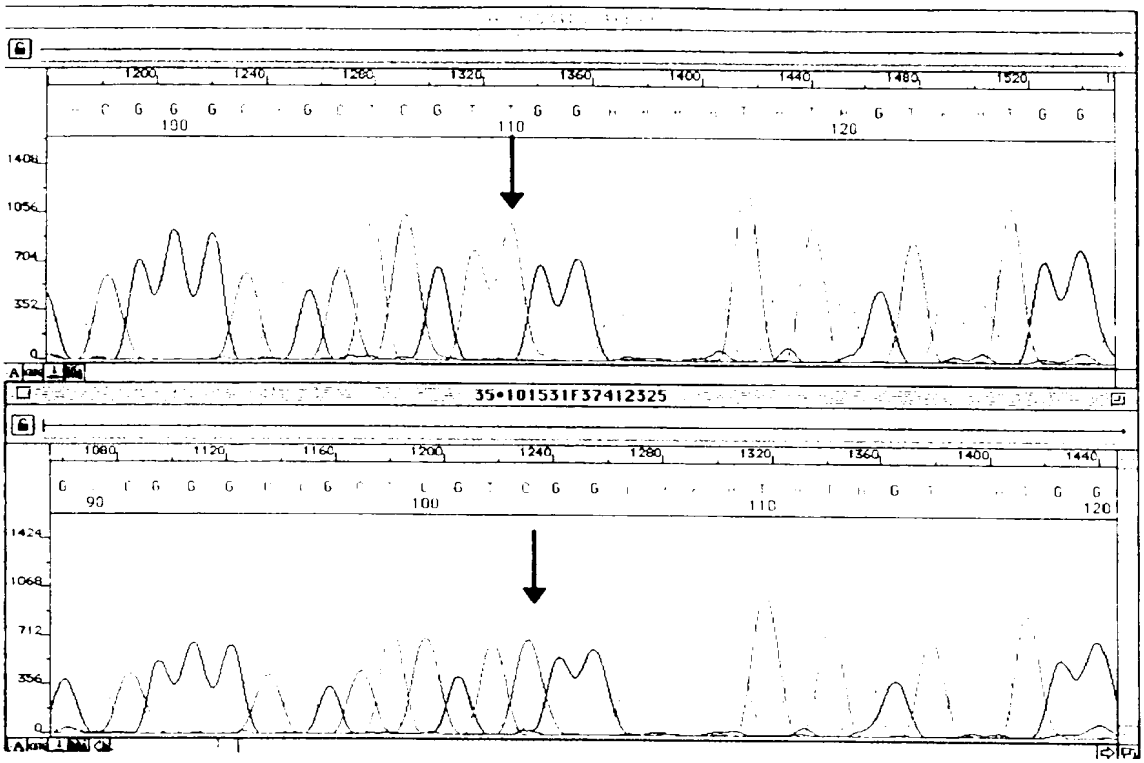
Wild Type (human foetal brain)	**T/T**
Variant Type (human adult brain)	**T/C**
Polymorphism Position	**3554**
RFLP	**-**

FIGURE 6



Wild Type (GM1416)	C/C
Variant (7225)	C/G
Position	4828

FIGURE 7



Primer sequences derived from BAC and used on lymphoblastoid cell lines from BPAD Patients.

Homozygous wild type (KK169) - T/T

Homozygous variant (KK232) - C/C

FIGURE 8

Figure 9

TGCTGCAAGTGACAGGTTCCAAGAAGCCCGAGGGCTCAGAGCTGAATGATGAAGCG
AGTCCCCAAAGTGCCTGGCCACCCCTCCCTCCCTGGATCACTGCTGCCTGGGCTTGA
TTGATTGATTGATTGATTGATTGATTGATTTTGAGAGAGATTCTCACTGTCACCCA
GCTGGAGTACAGTGGTGCGATCTCGGCTCACTGCAGCCTCTGCCTCCCGGGTTCAA
CAATTCTCCTGCCTCAGCCTCCCAAGTAGCTGGGACTACAGGCACGCGCCACCACA
CCAGCTAATTTTGTATTTTTAGTAAAAGACGGGGTTTCACCATGTTGGGCCAGGAT
GTCTTGATCTCCTGACCTCATGATCCACCCGCCCCGGCTTCCAAAGTGCTGGGATA
AGGCATGAACCCGACGCGCCCAGCATGGACATTTTTTTTTAATCCCCTGCCCTTTTC
TTGNGGCATAATTCATTGCAGGTCTCTTCTATACAGATCATGGAAAACACATTTTCT
TAACTGAGTTNTTATTATTTATACCCAGNCACCTCATGACANNTTTACCCTGTTACA
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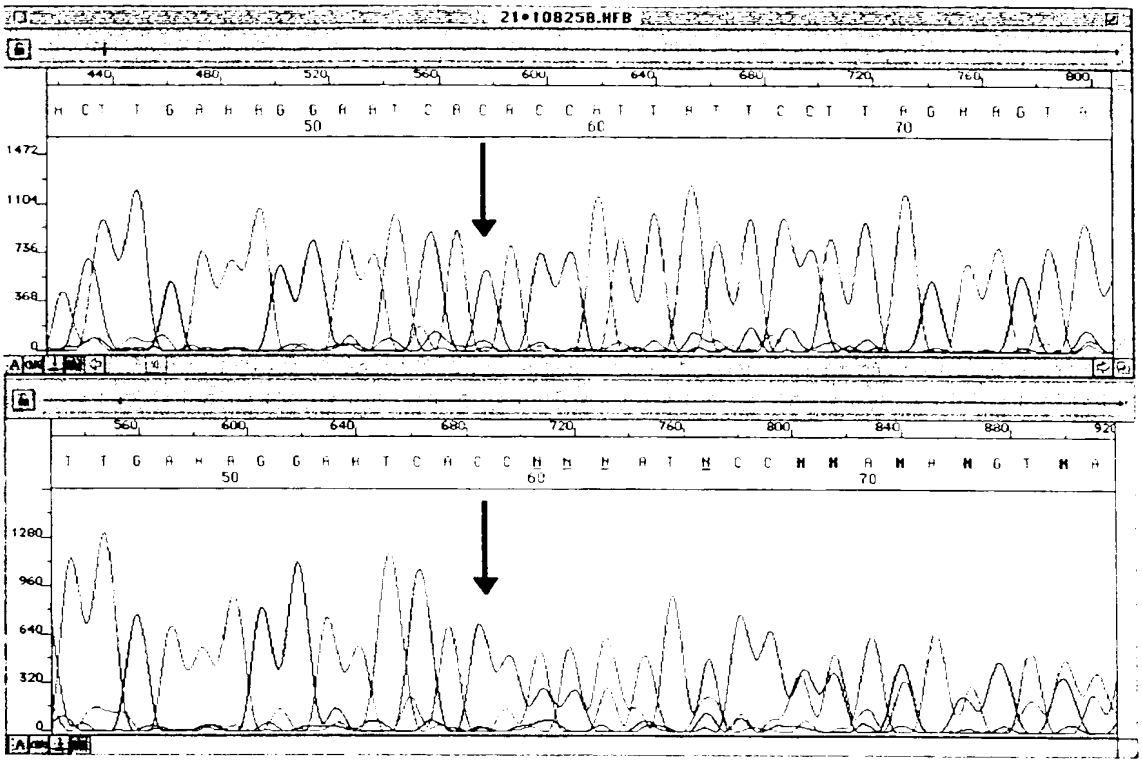
Tetranucleotide repeat underlined



Top electropherogram (human foetal brain) - wild type

Lower electropherogram (7225) - heterozygous variant

Arrow indicates the position of the C+C insertion - position 4032

FIGURE 10

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Zeneca Limited
(B) STREET: 15 Stanhope Gate
(C) CITY: London
(D) STATE: England
(E) COUNTRY: United Kingdom
(F) POSTAL CODE (ZIP): W1Y 6LN
(G) TELEPHONE: 0171 304 5000
(H) TELEFAX: 0171 304 5151
(I) TELEX: 0171 304 2042

(ii) TITLE OF INVENTION: NOVEL COMPOUNDS

(iii) NUMBER OF SEQUENCES: 5

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: GB 9716162.4
(B) FILING DATE: 01-AUG-1997

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5154 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CAAGCGCGCA ATTAACCCTC ACTAAAGGGA ACACCAACAC GTCGCCAGGA
CTGCGCCGTT 60

CGCTGCGCTC ATAGGCGGCG ATTTCATCAA GGGTGGCAAG GATCGCCTGG TCGACGGTCA 120

GGTCGTCCTC GACGCGGTTG CCCTCCTCGT CCTGTTCCAG GGTGAGTGGG CGATACCAGG 180

TGTCCACCGG GAAGGTACGG CCCGACACCT CGACAATCGG CGCATCGTCG AAGTGCTTGG 240

AAAAGCGCTC CAGGTCGATG GTGGCCGAGG TGATGATGAC TTTCAGGTCG GGGCGACGCG 300

GCAACAGGGT CTTGAGGTAG CCGAGCAGGA AGTCGATGTT CAGGCTGCGT TCGTGGGCTT 360

CGTCGACGAC AGGCTCGCGT TATGGCTCCG CTTTCTGCGG CTCTCCTACC CTGGCATGGT 420

GTGTGTGTGT GCCTGTGTGC TACGGAGAGT CCCGTATTCT CAGAGTAAAA GTTGTTCTGG 480

AATGATCTCG CCAAAAAGGA CATCTTTGGA GCCAGTGATC CGTATGTGAA ACTTTCATTG 540

TACGTAGCGG ATGAGAATAG AGAACTTGCT TTGGTCCAGA CAAAAACAAT TAAAAAGACA 600

CTGAACCCAA AATGGAATGA AGAATTTTAT TTCAGGGTAA ACCCATCTAA TCACAGACTC 660

CTATTTGAAG TATTTGACGA AAATAGACTG ACACGAGACG ACTTCCTGGG CCAGGTGGAC 720

GTGCCCCTTA GTCACCTTCC GACAGAAGAT CCAACCATGG AGCGACCCTA TACATTTAAG 780

GACTTTCTCC TCAGACCAAG AAGTCATAAG TCTCGAGTTA AGGGATTTTT GCGATTGAAA 840

ATGGCCTATA TGCCAAAAAA TGGAGGTCAA GATGAAGAAA ACAGTGACCA GAGGGATGAC 900

ATGGAGCATG GATGGGAAGT TGTTGACTCA AATGACTCGG CTTCTCAGCA CCAAGAGGAA 960

CTTCCTCCTC CTCCTCTGCC TCCCGGGTGG GAAGAAAAAG TGGACAATTT AGGCCGAACT 1020

TACTATGTCA ACCACAACAA CCGGACCACT CAGTGGCACA GACCAAGCCT GATGGACGTG 1080

TCCTCGGAGT CGGACAATAA CATCAGACAG ATCAACCAGG AGGCAGCACA CCGGCGCTTC 1140

CGCTCCCGCA GGCACATCAG CGAAGACTTG GAGCCCGAGC CCTCGGAGGG CGGGGATGTC 1200

CCCGAGCCTT GGGAGACCAT TTCAGAGGAA GTGAATATCG CTGGAGACTC TCTCGGTGTG 1260

GTTTTGCCCC CACCACCGGC CTCCCCAGGA TCTCGGACCA GCCCTCAGGA GCTGTCAGAG 1320

GAACTAAGCA GAAGGCTTCA GATCACTCCA GACTCCAATG GGGAACAGTT CAGCTCTTTG 1380

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CCAACAGTAA CTTTATTGCC CCGCTGGAGG GTGCCAAGGA CTCACCCGTA CGTCGGGCTG 1740

TGAAAGACAC CCTTTCCAAC CCACAGTCCC CACAGCCATC ACCTTACAAC TCCCCCAAAC 1800

CACAACACAA AGTCACACAG AGCTTCTTGC CACCCGGCTG GGAAATGAGG ATAGCGCCAA 1860

ACGGCCGGCC CTTCTTCATT GATCATAACA CAAAGACTAC AACCTGGGAA GATCCACGTT 1920

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AATGTGATGA 4380

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GGCCTGAGGT TTCTAAAAGA AGGTAGATTT CTACAGAGCT GAGTGTTGGT TCCTTTTTCT 5100

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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 975 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Phe Arg Leu Arg Ser Trp Ala Ser Ser Thr Thr Gly Ser Arg Tyr
1 5 10 15

Gly Ser Ala Phe Cys Gly Ser Pro Thr Leu Ala Trp Cys Val Cys Val
20 25 30

Pro Val Cys Tyr Gly Glu Ser Arg Ile Leu Arg Val Lys Val Val Ser
35 40 45

Gly Ile Asp Leu Ala Lys Lys Asp Ile Phe Gly Ala Ser Asp Pro Tyr
50 55 60

Val Lys Leu Ser Leu Tyr Val Ala Asp Glu Asn Arg Glu Leu Ala Leu
65 70 75 80

Val Gln Thr Lys Thr Ile Lys Lys Thr Leu Asn Pro Lys Trp Asn Glu
85 90 95

Glu Phe Tyr Phe Arg Val Asn Pro Ser Asn His Arg Leu Leu Phe Glu
100 105 110

Val Phe Asp Glu Asn Arg Leu Thr Arg Asp Asp Phe Leu Gly Gln Val
115 120 125

Asp Val Pro Leu Ser His Leu Pro Thr Glu Asp Pro Thr Met Glu Arg
130 135 140

Pro Tyr Thr Phe Lys Asp Phe Leu Leu Arg Pro Arg Ser His Lys Ser
145 150 155 160

Arg Val Lys Gly Phe Leu Arg Leu Lys Met Ala Tyr Met Pro Lys Asn
165 170 175

Gly Gly Gln Asp Glu Glu Asn Ser Asp Gln Arg Asp Asp Met Glu His
180 185 190

Gly Trp Glu Val Val Asp Ser Asn Asp Ser Ala Ser Gln His Gln Glu
195 200 205

Glu Leu Pro Pro Pro Pro Leu Pro Pro Gly Trp Glu Glu Lys Val Asp
210 215 220

Asn Leu Gly Arg Thr Tyr Tyr Val Asn His Asn Asn Arg Thr Thr Gln
225 230 235 240

Trp His Arg Pro Ser Leu Met Asp Val Ser Ser Glu Ser Asp Asn Asn
245 250 255

Ile Arg Gln Ile Asn Gln Glu Ala Ala His Arg Arg Phe Arg Ser Arg
260 265 270

Arg His Ile Ser Glu Asp Leu Glu Pro Glu Pro Ser Glu Gly Gly Asp
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Val Pro Glu Pro Trp Glu Thr Ile Ser Glu Glu Val Asn Ile Ala Gly
290 295 300

Asp Ser Leu Gly Val Val Leu Pro Pro Pro Pro Ala Ser Pro Gly Ser
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370 375 380

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Ser Pro Thr Val Thr Leu Xaa Ala Pro Leu Glu Gly Ala Lys Asp Ser
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Gln Pro Ser Pro Tyr Asn Ser Pro Lys Pro Gln His Lys Val Thr Gln
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565 570 575

Glu Asp Pro Arg Leu Gln Asn Pro Ala Ile Thr Gly Pro Ala Val Pro
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Tyr Ser Arg Glu Phe Lys Gln Lys Tyr Asp Tyr Phe Arg Lys Lys Leu
595 600 605

Lys Lys Pro Ala Asp Ile Pro Asn Arg Phe Glu Met Lys Leu His Arg
610 615 620

Asn Asn Ile Phe Glu Glu Ser Tyr Arg Arg Ile Met Ser Val Lys Arg
625 630 635 640

Pro Asp Val Leu Lys Ala Arg Leu Trp Ile Glu Phe Glu Ser Glu Lys
645 650 655

Gly Leu Asp Tyr Gly Gly Val Ala Arg Glu Trp Phe Phe Leu Leu Ser
660 665 670

Lys Glu Met Phe Asn Pro Tyr Tyr Gly Leu Phe Glu Tyr Ser Ala Thr
675 680 685

Asp Asn Tyr Thr Leu Gln Ile Asn Pro Asn Ser Gly Leu Cys Asn Glu
690 695 700

Asp His Leu Ser Tyr Phe Thr Phe Ile Gly Arg Val Ala Gly Leu Ala
705 710 715 720

Val Phe His Gly Lys Leu Leu Asp Gly Phe Phe Ile Arg Pro Phe Tyr
725 730 735

Lys Met Met Leu Gly Lys Gln Ile Thr Leu Asn Asp Met Glu Ser Val
740 745 750

Asp Ser Glu Tyr Tyr Asn Ser Leu Lys Trp Ile Leu Glu Asn Asp Pro
755 760 765

Thr Glu Leu Asp Leu Met Phe Cys Ile Asp Glu Glu Asn Phe Gly Gln
770 775 780

Thr Tyr Gln Val Asp Leu Lys Pro Asn Gly Ser Glu Ile Met Val Thr
785 790 795 800

Asn Glu Asn Lys Arg Glu Tyr Ile Asp Leu Val Ile Gln Trp Arg Phe
805 810 815

Val Asn Arg Val Gln Lys Gln Met Asn Ala Phe Leu Glu Gly Phe Thr
820 825 830

Glu Leu Leu Pro Ile Asp Leu Ile Lys Ile Phe Asp Glu Asn Glu Leu
835 840 845

Glu Leu Leu Met Cys Gly Leu Gly Asp Val Asp Val Asn Asp Trp Arg
850 855 860

Gln His Ser Ile Tyr Lys Asn Gly Tyr Cys Pro Asn His Pro Val Ile
865 870 875 880

Gln Trp Phe Trp Lys Ala Val Leu Leu Met Asp Ala Glu Lys Arg Ile
885 890 895

Arg Leu Leu Gln Phe Val Thr Gly Thr Ser Arg Val Pro Met Asn Gly
900 905 910

Phe Ala Glu Leu Tyr Gly Ser Asn Gly Pro Gln Leu Phe Thr Ile Glu
915 920 925

Gln Trp Gly Ser Pro Glu Lys Leu Pro Arg Ala His Thr Cys Phe Asn
930 935 940

Arg Leu Asp Leu Pro Pro Tyr Glu Thr Phe Glu Asp Leu Arg Glu Lys
945 950 955 960

Leu Leu Met Ala Val Glu Asn Ala Gln Gly Phe Glu Gly Val Asp
965 970 975

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 854 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ACAATGGGGG CGTGGCAGAG AATGGTTCTT CTTACTGTCC AAAGAGATGT TTAACCCCTA 60

CTATGGCCTC TTCGAGTACT CTGCCACGGA CAACTACACA CTTCAGATCA ATCCCAACTC 120

AGGCCTCTGT AATGAAGACC ATTTGTCCTA TTTCACCTTC ATTGGAAGAG TTGCTGGCCT 180

AGCGGTGTTT CATGGGAAAC TCTTAGATGG ATTCTTCATT CGACCATTCT ACAAGATGAT 240

GCTGGGGAAG CAGATAACGC TGAACGACAT GGAGTCCGTG GACAGCGAGT ACTACAACTC 300

TTTGAAGTGG ATCTTAGAAA ACGACCCCAC GGAACTTGAC CTCATGTTCT GCATAGACGA 360

GAGAACTTTG GGCAGACATA CCAAGTGGAT CTGAAGCCCA ACGGGTCAGA AATAATGGTA 420

ACCAATGAGA ACAAACGAGA ATACATTGAC TTAGTCATCC AGTGGAGATT TGTGAACAGG 480

GTCCAGAAGC AAATGAATGC CTTCTTGGAG GGATTTACAG AACTTCTTCC AATCGACTTG 540

ATTAAAATTT TTGATGAAAA TGAGCTGGAG TTGCTGATGT GCGGCCTTGG TGATGTCGAC 600

GTGAACGACT GGAGACAGCA CTCTATTTAC AAGAACGGCT ACTGCCCCAA CCACCCTGTC 660

ATCCAGTGGT TCTGGAAGGC CGTGCTCCTG ATGGATGCTG AGAAGCGCAT CCGGTTACTA 720

CAGTTTGTCA CAGGCACCTC CAGAGTACCC ATGAATGGAT TTGCCGAACT CTATGGTTCC 780

AATGGTCCTC AGCTGTTTAC AATAGAGCAA TGGGGCAGTC CGAAAAACTA CCAGAGCTCT 840

ACATGCTTAA TCGC 854

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 604 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

His Ala Cys Ser Asn Ala Ala Ser Arg Ala Ala Ala Arg Val Ala Ala
1 5 10 15

Arg Cys Thr Ala Arg Ser Arg Ser Gly Arg Arg Ser Ser Ser Val Ser
20 25 30

Arg Ser Ser Ser Arg Gly Ala Ser Ser Ser Met Ser Ser Asp Met Ala
35 40 45

Ala Asp Ser Ala Val Ser Asp Val Trp Cys Asp Lys Thr Asp Gly Gly
50 55 60

Gly Ser Gly Ser Asp Val Thr Asp Thr Cys Cys Gly Cys Trp Asn Asn
65 70 75 80

Ser His Val Thr Ala Asp Tyr His Asn Asp Asp Thr Arg Val Val Arg
85 90 95

Val Lys Val Ala Gly Gly Ala Lys Lys Asp Gly Ala Ser Asp Tyr Val
100 105 110

Arg Val Thr Tyr Asp Met Ser Gly Thr Ser Val Thr Lys Thr Lys Lys
115 120 125

Ser Asn Lys Trp Asn Arg Val Arg His Arg Val Asp Asn Arg Thr Arg
130 135 140

Asp Asp Gly Val Asp Val Tyr Thr Asn Arg Met Arg Tyr Thr Lys Asp
145 150 155 160

Val His Arg Ser His Lys Ser Arg Val Lys Gly Tyr Arg Lys Met Thr
165 170 175

Tyr Lys Asn Gly Ser Asp Asn Ala Asp Ala Gly Trp Val Val Asp Asp
180 185 190

Ala Ala Thr His His Ser Gly Trp Arg Asp Val Gly Arg Thr Tyr Tyr
195 200 205

Val Asn His Ser Arg Arg Thr Trp Lys Arg Ser Asp Asp Asp Thr Asp
210 215 220

Asp Asn Asp Asp Met Ala Arg Ala Thr Thr Arg Arg Ser Asp Val Asp
225 230 235 240

Gly Asp Asn Arg Ser Asn Trp Val Arg Asp Asn Thr Tyr Ser Gly Ala
245 250 255

Val Ser Ser Gly His Asp Val Thr His Ala Asn Thr Arg Ala Val Cys
260 265 270

Gly Asn Ala Thr Ser Val Thr Ser Ser Asn His Ser Ser Arg Gly Gly
275 280 285

Ser Thr Cys Thr Val Thr Ser Ser Gly Gly Trp Lys Asp Asp Arg Gly
290 295 300

Arg Ser Tyr Tyr Val Asp His Asn Ser Lys Thr Thr Thr Trp Ser Lys
305 310 315 320

Thr Met Asp Asp Arg Ser Lys Ala His Arg Gly Lys Thr Asp Ser Asn
325 330 335

Asp Gly Gly Trp Arg Thr His Thr Asp Gly Arg Val Asn His Asn Lys
340 345 350

Lys Thr Trp Asp Arg Asn Val Ala Thr Gly Ala Val Tyr Ser Arg Asp
355 360 365

Tyr Lys Arg Lys Tyr Arg Arg Lys Lys Lys Thr Asp Asn Lys Met Lys
370 375 380

Arg Arg Ala Asn Asp Ser Tyr Arg Arg Met Gly Val Lys Arg Ala Asp
385 390 395 400

Lys Ala Arg Trp Asp Gly Lys Gly Asp Tyr Gly Gly Val Ala Arg Trp
405 410 415

Ser Lys Met Asn Tyr Tyr Gly Tyr Ser Ala Thr Asp Asn Tyr Thr Asn
420 425 430

Asn Ser Gly Cys Asn Asp His Ser Tyr Lys Gly Arg Val Ala Gly Met
435 440 445

Ala Val Tyr His Gly Lys Asp Gly Arg Tyr Lys Met Met Lys Thr His
450 455 460

Asp Met Ser Val Asp Ser Tyr Tyr Ser Ser Arg Trp Asn Asp Thr Asp
465 470 475 480

Arg Asp Gly Thr His His Lys Thr Gly Gly Ser Val Val Thr Asn Lys
485 490 495

Asn Lys Lys Tyr Tyr Val Trp Arg Val Asn Arg Lys Met Ala Ala Lys
500 505 510

Gly Asp Lys Asp Asn Met Cys Gly Gly Asp Val Asp Val Asn Asp Trp
515 520 525

Arg His Thr Lys Tyr Lys Asn Gly Tyr Ser Met Asn His Val His Trp
530 535 540

Trp Lys Ala Val Trp Met Met Asp Ser Lys Arg Arg Val Thr Gly Thr
545 550 555 560

Xaa Ser Arg Val Met Asn Gly Ala Tyr Gly Ser Asn Gly Ser Thr Val
565 570 575

Trp Gly Thr Asp Lys Arg Ala His Thr Cys Asn Arg Asp Tyr Ser Asp
580 585 590

Trp Asp Lys Met Ala Asn Thr Gly Asp Gly Val Asp
595 600

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 615 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

TGCTGCAAGT GACAGGTTCC AAGAAGCCCG AGGGCTCAGA GCTGAATGAT GAAGCGCAGT 60

CCCCAAAGTG CCTGGCCACC CCTCCCTCCC TGGATCACTG CTGCCTGGGC TTGATTGATT 120

GATTGATTGA TTGATTGATT GATTTTGAGA GAGATTCTCA CTGTCACCCA GGCTGGAGTA 180

CAGTGGTGCG ATCTCGGCTC ACTGCAGCCT CTGCCTCCCG GGTTCAAGCA ATTCTCCTGC 240

CTCAGCCTCC CAAGTAGCTG GGACTACAGG CACGCGCCAC CACACCCAGC TAATTTTGTA 300

TTTTTAGTAA AAGACGGGGT TTCACCATGT TGGGCCAGGA TGGTCTTGAT CTCCTGACCT 360

CATGATCCAC CCGCCCCGGC TTCCAAAGTG CTGGGATACA GGCATGAACC CGACGCGCCC 420

AGCATGGACA TTTTTTTTTA ATCCCCTGCC CTTTTCTTGG GCATAATTCA TTGCAGGTCT 480

CTTCTATACA GATCATGGAA AACACATTTT CTTAACTGAG TTTTATTATT TATACCCAGC 540

ACCTCATGAC ATTTACCCTG TTACAACAAA ATGGGCACCT GCCAAAACAA CTTTATATAA 600

GGATGCTCCA GGCCT 615

INTERNATIONAL SEARCH REPORT

Internat. Application No
PCT/GB 98/02259

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/00 C07K14/435 C12N9/10 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X,P	WO 97 37223 A (UNIV NORTH CAROLINA) 9 October 1997	6,10, 12-14, 18-21
A	see abstract see page 9, line 1 - page 10, line 23 see figure 23 see claim 48 see Nos.125 and 126 of Sequence Listing ---	1,2,4
X,P	OHARA O. ET AL.: "Prediction of the sequences of unidentified human genes. VIII. The complete senquences of 77 new cDNA clones from brain which can code for large proteins in vitro" EMBL DATABASE,5 December 1997, XP002087609 HEIDELBERG, DE AC: AB007899 --- -/--	1,2,4, 8-10,18, 21

[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 December 1998	12/01/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Panzica, G

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INTERNATIONAL SEARCH REPORT

Internat ... Application No
PCT/GB 98/02259

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	STINE O.C. ET AL.: "Evidence for linkage of bipolar disorder to chromosome 18 with a parent-of-origin effect" AMERICAN JOURNAL OF HUMAN GENETICS, vol. 57, no. 6, 1995, pages 1384-1394, XP002087610 US cited in the application see the whole document	
A	MORS O. ET AL.: "Cytogenetic abnormalities on chromosome 18 associated with bipolar affective disorder or schizophrenia" BRITISH JOURNAL OF PSYCHIATRY, vol. 170, March 1997, pages 278-280, XP002087611 GB	

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat l Application No
PCT/GB 98/02259

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9737223 A	09-10-1997	AU 2659797 A	22-10-1997